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NAME OF AUTHOR Pliny Harold Hayes

TITLE OF THESIS Mutant Analysis of Determination in *Drosophila*

DEGREE FOR WHICH THESIS WAS PRESENTED Doctor of Philosophy

YEAR THIS DEGREE GRANTED Fall 1982

THE UNIVERSITY OF ALBERTA

Mutant Analysis of Determination in *Drosophila*

by

Pliny Harold Hayes

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF Doctor of Philosophy

Department of Genetics

EDMONTON, ALBERTA

Fall 1982

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled Mutant Analysis of Determination in *Drosophila* submitted by Pliny Harold Hayes in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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To my parents and my brothers.

Abstract

This thesis concerns the interpretation of homeotic mutants in *Drosophila melanogaster*. Various systems of gene interaction have been proposed as the basis for determined states in *Drosophila* development. Three are defined here in a rigorous fashion, and the expected effects of mutants within each system as well as the effects of mutant combinations are examined. It is argued that actual developmental systems employ a mixture of the different pure systems. The *engrailed* mutation and various mutants in the proximal Bithorax Complex are considered in light of the theory which has been developed, and specific conclusions are drawn concerning the control of gene activation and the interactions among those genes to give the final determined state.

> "We make a wonder at the monstrous and mighty shoulders of Elephants, able to carry turrets upon them. We marvell at the strong and stiffe necks of buls, and to see how terribly they will take up things and tosse them aloft into the aire with their hornes. We keepe a wondering at the ravening of Tygres, and in the shag manes of Lions: and yet in comparison of these Insects there is nothing wherein Nature and her whole power is more seene, neither sheweth she her might more than in the least creatures of all. I would request therfore the Readers, that in perusing this treatise, they will not come with a prejudicate opinion, nor (because many of these silly flies and wormes be contemptible in their eies) disdaine, loath, and contemne the reports that I shall make thereof; seeing there is nothing either in Natures workes that may seeme superfluous, or in her order unworthy our speculation."

-- Pliny, *Natural History*, Book XI

Acknowledgements

It is a great pleasure to acknowledge my deep gratitude to Michael Russell for his help and encouragement, his friendship and patience. I am grateful to Stanley Tiong, John Williams, Jim Kennison, Emil Steiner, Sue Eberlein, and Asma Zaidi for their friendship and for very helpful comments on various aspects of the thesis. For their friendship, example, and encouragement, I thank Chris and Shauna Somerville, Marlene Johnstone, Brian Golding, Siew-Keen Quah, Effie Woloshyn, Curt Strobeck, Ken Morgan, Mary Holmes, Elizabeth Savage, and Maureen McCall.

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I. Introduction

This thesis is an inquiry into the genetic control of determination in *Drosophila*. In essence, it attempts to answer the question "How does one properly interpret homeotic mutants?" That there is no consensus is clear from the large number and variety of models which have been constructed around essentially the same data base. In general, the models which have been proposed attempt to explain all homeotic mutants with a single rationale. I believe that this attempt is misguided: while each approach has features which recommend it, none is sufficient alone, for reasons to be developed in the text. In this thesis I develop a common terminology by which different rationales are compared and integrated. Chapters II and III are concerned with the properties of developing systems in general and those specific to *Drosophila*. Chapters IV and V present a theoretical analysis of different mutant interaction systems and the properties expected of mutants in each system. Chapters VI and VII describe and analyse specific mutations in *Drosophila melanogaster*. Chapter VIII is concerned with the interpretation of mutant combinations, Chapter IX with deletions, and Chapter X summarizes the conclusions of the thesis.

II. Positional Information and Genetic Response

A. Positional Information

Developmental systems may be classified as "mosaic" or "regulative" depending upon the behavior of isolated fragments of the system under conditions conducive to further development. In mosaic systems, fragments differentiate precisely the set of structures to which they would have given rise *in situ*. The fragments are thus self-differentiating. In regulative systems, fragments give rise to more structures ("positive regulation") or fewer structures ("negative regulation") than would have been found *in situ* (Sander, 1971). Regulative behavior suggests that the fragment forms part of a larger system which requires communication between the parts for the establishment of a normal pattern. Regions with this property have been termed "fields" (see Weiss, 1939). Patterns of regulation in fragments of fields or in fusions of similar fields have been extensively studied to determine the nature of the underlying interactive system (see Kuhn, 1971, for a detailed survey of 80 years of experimental embryology). In general it is found that many results may be explained if a gradient is invoked in the process of pattern specification. Thus, fields as defined operationally by a variety of experimental criteria may correspond to the limits of gradient systems.

The early twentieth century saw the emergence of the field of genetics, and investigations into the roles of genes in development. From the results of studies of genetic mosaics, Stern proposed that the terminal morphology of a structure arose from an interplay between singularities in an underlying "prepattern" and the genetic competence to react of the cells in the field (Stern, 1968). It was not until the late sixties, however, that a unifying theoretical basis for the actions of genes and gradients was proposed, when Wolpert made rigorous the concept of positional information (Wolpert, 1969).

Wolpert proposed that graded information exists in fields, but the information relates strictly to position. The field as revealed operationally is now proposed to comprise those cells whose positions are defined with respect to the same set of co-ordinates. During development, each cell would (i) read its position within a field, and (ii) interpret it by activating an appropriate set of genes to give a specific differentiated

state. The unique properties of a given field are thus thought to result from singularities in the cellular response rather than from singularities in the underlying positional information.

Wolpert points out that fields are never more than one hundred cells in diameter (the upper limit perhaps being due to the limitations of diffusion), and this may explain why in organisms larger than this fields became subdivided into secondary fields during development (Weiss, 1939). Wolpert also suggests that a single mechanism may be used to specify position in different organisms or within different secondary fields in a single organism if the characteristics that distinguish different fields are built into the genetic responses to positional information. There is ample evidence that, at the very least, this latter point is correct: experiments with vertebrate limb buds (Saunders, *et al.,* 1957), the insect integument (Locke, 1966; Stumpf, 1967; Lawrence, 1966), and *Drosophila* mutants (Postlethwaite and Schneiderman, 1971; Struhl, 1981), morphogenetic mosaics (Stern, 1968) and imaginal discs (Wilcox and Smith, 1977; Bryant *et al.,* 1978; Adler, 1978a) have shown that cells which normally reside in different secondary fields can respond to each other's positional specification coherently (French, *et al.,* 1976) but autonomously according to their differing genotypes or developmental histories.

B. Genetic Response to Positional Information

At precise moments in the developmental program continuous fields became subdivided into discrete developmental subunits (Weiss, 1939; Garcia-Bellido, 1975a). What can be said about the nature of the genetic events underlying this transition? A very few cases have been elucidated in which developmental restrictions are accompanied by a loss of DNA (e.g. *Ascaris,* Bovari, 1899). This is in general not the case however, since transplantation experiments have shown that isolated nuclei from various stages of development of a vertebrate (Gurdon, 1962) and an insect (Illmensee, 1976) can support development of a fertile adult when injected into enucleate eggs. Genetic models of development have thus focused on ways of turning genes on in specific locations.

One type of model which has been proposed makes use of allosteric proteins to monitor position in a field and direct different pathways of development (e.g. Kiger, 1973). Just as the haemoglobin molecule changes shape with differing oxygen tensions,

the hypothetical allosteric protein would assume various allosteric configurations in response to levels of a graded "morphogen," changing morphs at particular threshold values. Each configuration would then direct a specific developmental pathway by activation of a specific set of "downstream" genes (or "batteries of genes," Britten and Davidson, 1969). However, in larger organisms the secondary fields develop autonomously and yet retain a memory of their position within the primary field. The existence of this cellular memory essentially disqualifies models based upon allosteric proteins alone, for the persistence of shape differences in allosteric proteins would require the persistence of different levels of morphogen (i.e. the primary gradient). Thus, if allosteric proteins are involved in the subdivision of fields, their roles must be limited to that of intermediaries, translating the information in transient gradients into a clonally stable state such as those suggested below (see Lewis, *et al.*, 1977, for discussion).

A second class of model has been proposed which is characterized by developmental control genes ("switch genes", Waddington, 1966; "binary switches", Kauffman, 1973; "selector genes", Garcia-Bellido, 1975a). These genes would be set in an ON or OFF state depending upon position within a field, and would remain stably ON or OFF in the cell and its clonal descendents when once so set. Between them they would control access to the various developmental pathways (see below, section 4), again by activating "downstream" genes. Lewis, Slack, and Wolpert (1977) have discussed in detail one system with the requisite mitotic stability. They envision a gene activated by a graded morphogen; transcription of the gene would be promoted in a linear fashion by the morphogen and in a sigmoidal fashion by the gene product. The result is that above a threshold value the gene will be continuously transcribed, even after the disappearance of the gradient. A second method of ensuring stability in developmental systems has been suggested by Holliday and Pugh (1977). It is based on recent findings regarding the extent, distribution, and stability of 5-methyl-cytosine in DNA, and the fact that the set of transcriptionally active genes appears to be undermethylated with respect to the whole genome (see Ehrlich and Wang, 1981, for review). Sequence specific methylases (or demethylases as seems more likely in view of recent evidence) are envisioned initially to determine the set of genes which is transcriptionally active, and maintenance methylases acting at DNA replication at hemi-methylated sites would ensure mitotic

stability.

The control gene and the methylase models share two formal properties: position specific gene activation and a provision ensuring clonal memory. The two models may be distinguished: the positive feedback loop characteristic of the control gene model results in the memory component residing at the same locus as the positional specificity; in the methylase model two different genes contribute these components. Note that whatever the nature of the memory component, it cannot be an irreversible alteration of the DNA, for the nuclear transplantation experiments mentioned above show that under appropriate conditions a gene may be turned off after once being activated.

III. Drosophila Development

The specific organism discussed in this thesis is *Drosophila melanogaster*. In this chapter I describe the course of early development in *Drosophila* with special reference to prospective adult epidermal cells, as these tissues supply most of the available data concerning the control of development in *Drosophila*.

A. Descriptive Embryology

After fertilization, a number of rapid nuclear divisions occur without cytokinesis, forming a syncytium. For the first eight divisions the cleavage energids remain in the yolky interior of the egg, after which most of the nuclei migrate to the periplasm (Huettner, 1923) where they divide another five times. By 3.5 hours after oviposition cell membranes have developed around the nuclei and a cellular blastoderm of approximately 6,000 cells has been formed (Zalokar, 1975; Turner and Mahowald, 1976). At 3.5 hours, gastrulation begins, and by five hours the first visible signs of segmentation have appeared (Turner and Mahowald, 1977). The fully segmented first instar larva hatches from the egg approximately 22 hours after oviposition.

As *Drosophila* is a holometabolous insect, the adult and larval cuticular structures are formed from separate cell lineages. The adult cell lines remain diploid while larval cells become increasingly polyploid during development. The adult cuticular structures of the head, thorax, and genitalia are derived from invaginated groups of cells called imaginal discs which are first visible in the newly hatched larva (Auerbach, 1936; Madhavan and Schneiderman, 1977). By clonal analysis (see below) it has been determined that an average of one cell division occurs between three and ten hours after oviposition in a number of imaginal discs (Wieschaus and Gehring, 1976); cell division then ceases and is not resumed until sometime later in the first larval instar (Bryant and Schneiderman, 1969; Bryant, 1970; Postlethwait and Schneiderman, 1971). These conclusions have been confirmed by a direct cytological analysis (Madhavan and Schneiderman, 1977).

The adult cuticular structures of the abdominal segments are derived from specific groups of cells imbedded in the larval epithelium called histoblast nests. Each

segment contains two dorsal histoblast nests which give rise to the adult tergite and a single ventral histoblast nest which forms the sternite. At 17 hours after hatching, the anterior and posterior dorsal histoblast nests contain approximately 12 – 17 and 4 – 8 cells respectively and the ventral nest contains approximately 11 – 14; these numbers remain constant throughout larval life until five hours after pupariation when rapid mitoses generate the requisite number of cells for the adult structures. Although adults of both sexes lack a first abdominal sternite and males lack a seventh abdominal tergite and sternite, the corresponding histoblast nests are present in the larva (Madhavan and Schneiderman, 1977).

The progenitor cells of the adult structures have been localized on the blastoderm surface by the use of genetic mosaics ("gynandromorph mapping", see Janning, 1978) and by the correlation of site specific damage at blastoderm with subsequent adult defects (see Lohs-Schardin, *et al.*, 1979). The results of the two techniques are consistent, and reveal that the primordia of adult cuticular structures are located in very specific regions of the blastoderm surface.

The results of a third technique, clonal analysis (Stern, 1936), extend our understanding of the process by which adult-specific blastoderm cells acquire their fates. When cells are irradiated, somatic recombination occurs at low frequency. If the cells in question are heterozygous for a mutation affecting cellular phenotype, such events may result in the segregation of a homozygous mutant cell at the next mitosis. The clonal descendants of such a cell will also be homozygous, and will be recognizable by their mutant phenotype in a wild-type background. If the cell marker mutation is combined appropriately with a mutation affecting cell division rate, very large clones of fast growing mutant cells may be generated (Morata and Ripoll, 1975). When *Drosophila* embryos at the cellular blastoderm stage were irradiated, the clonal descendents of individual blastoderm cells were seen to be limited in the array of adult structures they could form. No clone was observed to cross a boundary between segments. In addition, clones did not cross between anterior and posterior regions of particular segments, even though they were seen to mark both dorsal and ventral structures within the segment (e.g. wing and second leg, haltere and third leg) (Steiner, 1976; Wieschaus and Gehring, 1976). As nuclei appear to be totipotent before cellularization occurs (Illmensee, 1978), these

lines of clonal restriction reveal the first steps in *Drosophila* determination. Irradiation during subsequent development reveals that further lines of clonal restriction are formed in a specific sequence, first separating derivatives of individual discs, and then dorsal from ventral, proximal from distal, etc. in specific discs. Regions bounded by lines of clonal restriction have been termed "compartments" (Garcia–Bellido, Ripoll, and Morata, 1973, 1976).

B. Results of Experimental Intervention

In a series of experiments on various Diptera, embryos were ligated in a transverse fashion at various times before cellular blastoderm and at varying anterio–posterior levels (reviewed in Sander, 1975a). It was observed that the resulting partial embryos contained an incomplete number of segments. The size of the "gap" decreased with increasing age of the embryo until the cellular blastoderm stage, when the missing structures were limited to those normally formed at the site of the constriction. In addition it was found that when previously separated portions of ligated embryos were made to fuse secondarily after as many as ten hours separation, the normal pattern of development could be restored. The restoration of missing segments indicates that the initially incomplete pattern was not due to the destruction of localized determinants but rather to the inhibition of communication between the separated embryonic regions. The partial embryos thus show negative regulation, and the embryo behaves as a field. The gap phenomenon has also been observed in ligation experiments on *Drosophila* embryos (Schubiger and Wood, 1977), as has the restoration of normal development upon the re–establishment of communication (Schubiger, Mosely, and Wood, 1977).

Further evidence for non–mosaic determination in insects comes from experiments with the leaf hopper *Euscelis*, in which polar cytoplasm may be readily identified by the presence of a symbiont ball. Upon transverse ligation of *Euscelis* embryos, anterior fragments produce only head segments and posterior fragments produce terminal abdominal segments. However, when posterior cytoplasm is mechanically displaced to a medial position prior to ligation, the pattern within ligation fragments is altered in ways dependent upon experimental conditions. In extreme cases a complete embryo may be formed in the anterior fragment and a duplicate pattern of

posterior terminal segments in mirror image symmetry in the posterior. In less extreme cases the anterior fragment may contain an incomplete set of posterior segments, and the posterior fragment may contain a single set of posterior terminal segments in reversed polarity (Sander, 1960). Two features of the results are inconsistent with mosaic determination: (i) posterior cytoplasm may be associated with the establishment of more than one state of determination (i.e. terminal *in situ*, subterminal upon displacement), and (ii) segments are always observed to be evenly spaced within fragments although their absolute size may vary greatly. Both features are explained by a model in which segmental pattern formation is controlled by the interaction of opposing gradients (Sander, 1960).

C. Mutant Effects in Development

A great many mutations affecting development have been isolated in *Drosophila melanogaster*. They are of interest because they may identify components of positional information systems discussed in Chapter II. They may conveniently be divided into three categories: those with maternal effects, those affecting either the number or polarity of segments, and those affecting the determination of segments or compartments.

Two maternal effect mutants have been isolated which result in mirror image symmetry in the larval pattern of segmentation. Expressivity of the *bicaudal* mutation varies greatly, but in extreme cases embryos from *bicaudal* mothers contain mirror symmetric arrangements of a varying number of abdominal segments (Bull, 1966; Nusslein–Volhard, 1977). Analogous mirror symmetric arrangements of dorsal structures may be seen in the *dorsal* mutation (Nusslein–Volhard, 1979). The phenotypes and maternal action of these mutants suggest that they affect the spatial co–ordinates of the embryo, and *bicaudal* and *dorsal* have been interpreted as evidence for the action of anterio–posterior and dorso–ventral gradients of positional information in *Drosophila* development (Nusslein–Volhard, 1979).

Mutants affecting the number and polarity of segments were recently isolated by Nusslein–Volhard and Wieschaus (1980) from a screen of mutations causing embryonic lethality. They were found to cause three different types of pattern abnormality. One class of mutants defining three loci results in the absence of specific sets of contiguous

segments. A second class of six loci contained varied phenotypes, but each could be ascribed to a polarity reversal within a repeating unit the length of a single segment. The third class, with six loci, consisted of mutations causing deletions in different homologous regions of alternating segments. The finding of this latter class was unexpected; it is most easily explained if pairs of segments are treated as single units which are homologously affected. This suggests the existence of a transient stage in development when the embryo is organized in repeating units of two segments (Nusslein–Volhard and Weischaus, 1980). Combinations of mutations in this latter class with other mutations affecting segmental identity (see below) have revealed that it is the position of segments within the segmental array rather than the identity of segments which determines the pairs of segments which behave as units.

Various mutations affect determination in *Drosophila* in that they cause the often spectacular transformations of one body part into another (i.e. antennae into legs). A large part of this thesis will discuss particular homeotic mutants in detail; here I wish merely to point out that the effects of many homeotic mutants are segment or compartment–specific. They thus identify loci which may correspond to the developmental control genes which respond to gradients of positional information.

IV. Integration of Information from Different Fields

To this point I have been concerned with the existence and developmental control of genes which specify particular pathways. In this chapter, I consider the possible interactions among such control genes which may result in determined states. Various models have been proposed to explain this aspect of *Drosophila* development. These models may be conveniently divided into two classes depending upon the way the determined state is coded. In the first class of model, commonly referred to as "combinatorial", the ON/OFF activity states of a set of globally critical genes uniquely encode each determined state. The second class of model I shall call "ideographic"; it is characterized by the association of genes with particular levels of development, which results in some degree of degeneracy in an underlying combinatorial code. Two distinct types of ideographic models are possible, differing in the way control genes interact to give determined states. Each model will be considered in isolation, followed by a discussion of mixed systems. Chapter V examines the different expected behavior of mutants in the different systems.

A. Combinatorial Systems

As revealed by clonal analysis, the blastodermal determinative events appear to differ from the subsequent compartmentalization events in discs in that the early events subdivide the egg simultaneously into many discrete units while the later determinative events in discs are binary. The apparent difference may be an artifact of the somatic crossover technique, however, for while the initial events are revealed by a single X–irradiation at the blastoderm stage, they could occur at any time between blastoderm and the onset of mitosis approximately seven hours later. Thus, what appear to be simultaneous events coud be heirarchically ordered. Kauffman (1973) has proposed that the events leading to segmental establishment and determination are also in fact binary, bisecting fields by the activation of a "binary switch" (control gene) in one of two sub–regions. A series of such events –– each subdividing all regions formed to that point –– results in segments being uniquely distinguished by the combination of ON/OFF states

of all binary switches (see Figure 4-1). Evidence supporting the model comes from three independent sources, an analysis of lethal mutations affecting imaginal discs, the frequencies and directions of transdetermination events, and some of the mutations in segmentation mentioned above.

A number of late larval lethals causing abnormalities in discs was isolated by Shearn,*et al.*, (1971). When categorized by the specific discs affected, the mutants fell into thirteen classes, eight of which formed complementary pairs. Significantly, when the locations of the disc primordia on the blastoderm surface were considered, the complementary subsets could be separated by a geometrically simple pattern of lines. This suggested to Kauffman that each subset might be distinguished from its complement by the differing ON/OFF state of a "binary switch" triggered by position in the embryonic field. "Transdetermination" is the name given to the phenomenon in which disc blastemas in culture spontaneously change their states of determination (Hadorn, 1965). It is known that each determined state transdetermines at characteristic frequencies to other states. Each step is reversible, but because of oriented frequency differences a global tendency to transdetermine towards mesonotum is observed. Kauffman (1973) found that by consistently assuming different stability properties in ON and OFF states of binary switches he could assign specific four-digit binary numbers to each determined state that would account for most of the preferred directions of transdetermination events. These four digit numbers could be chosen such that they were also compatible with the complementary pairs among Shearn's lethals. Finally, a number of mutants isolated as embryonic lethals result in half the normal number of segments (Wieschaus and Nusslein-Volhard, 1980). While other explanations are possible, the phenotypes of these mutants are consistent with the existence of a transient developmental unit consisting of an adjacent pair of segments, as predicted by a combinatorial model.

Garcia-Bellido has applied this same approach to the post-blastoderm compartmentalization events in discs in his "selector gene" model (Garcia-Bellido, 1975). Noting that compartmentalization events always bisect existing compartments, he proposed that in such events a selector gene (control gene) is stably activated in one of the two regions. The differing activity states of the gene would maintain the distinctions between the compartments throughout development and account for their different

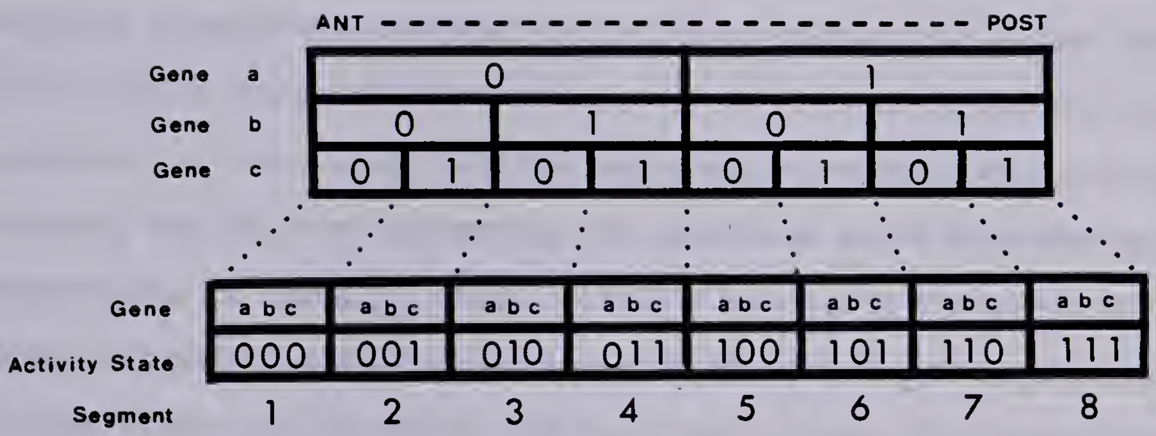


Figure 4-1. A linear array of eight segments delineated and uniquely determined by the ON/OFF states of three genes: in this and succeeding figures the following convention is used: 1 = gene ON; 0 = gene OFF. The upper figure shows the activity states of three control genes within the embryo. The lower figure shows the combined activity states of the three genes in each segment (after Kauffman).

determination. Homologous decisions in equivalent secondary fields are posited to be recorded by the ON/OFF states of the same selector gene, e.g. the anterior and posterior compartments of all thoracic discs are posited to be distinguished by the OFF and ON states respectively of the *engrailed* gene. In both the selector gene model and the binary switch model, therefore, the terminal determined states are associated with a unique combination of states at a set of control loci.

B. Ideographic Models

In ideographic models, more than one set of control locus states may result in the same state of determination. Codes of this type are termed "degenerate". An example of such a model is Lewis' (1978) interpretation of the action of the Bithorax Complex. As opposed to Kauffman, Lewis feels that segmentation and determination are separate processes, and that after segmentation the metathorax and all abdominal segments are determined by the states of control loci initially activated by a primary longitudinal gradient, the genes being activated when gradient levels exceed successively higher threshold values. This results in an ordered series of gene activity states similar to that depicted in Figure 4–2. As Lewis notes (loc. cit.), the determined states may be derived from these activity states in two different ways, depending upon the nature of the interactions between genes. In what I shall call a "cumulative" model, each gene would act to raise the developmental level by a single step from that of the next most anterior segment to that of the segment the gene specifies. Here, a gene would be necessary but not sufficient to determine the level of development it specifies; in addition, that state of determination would require the activity of all the control genes with lower thresholds. As the ON/OFF state of a gene does not contribute to the determined state in cases where these specific preconditions are not met, cumulative models are necessarily degenerate (see Figure 4–2a).

In what will be referred to as an "epistatic" model (see Figure 4–2b), each control gene would override the effects of all others with lower activation thresholds and would raise the level of development from the "developmental sink" (0000) directly to that of the segment which it determines. As a consequence of this kind of "epistatic" interaction, the ON/OFF states of genes which are overridden may vary without affecting the

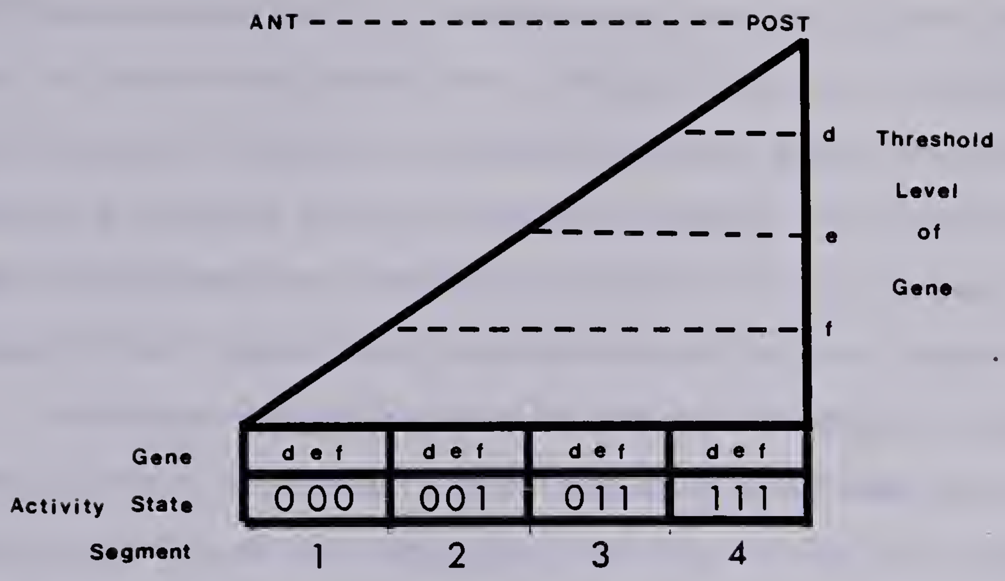


Figure 4-2. A linear array of four segments distinguished by the activity states of three genes activated by different thresholds of a linear gradient. Note that only four of the eight possible activity state combinations are used in the wild-type.

Segment	1	2	3	4
Gene	d e f	d e f	d e f	d e f
Wildtype Code	000	001	011	111
Alternate Codes	010 100 110	101		

Figure 4-2a. The assignments of the remaining four activity state combinations in the cumulative model. Each gene requires the ON state of all genes with lower thresholds to be active in determination.

	1	2	3	4
Gene	d e f	d e f	d e f	d e f
Wildtvpe Code	000	001	011	111
Alternate Codes			010	100 101 110

Figure 4-2b. The assignments of the remaining four activity state combinations in the epistatic model. Each gene requires the OFF state of all genes with higher thresholds to be active in determination.

determined state, also resulting in a degenerate code (see Figure 4–2b). Evidence in support of Lewis' model comes from an analysis of mutants in the Bithorax Complex, and will be discussed in Chapter VI. Ideographic models in general may differ in the pattern of control gene activation, and may include both "epistatic" and "cumulative" elements. They may be distinguished from combinatorial models in that control genes are associated with particular levels of development, and the codes are therefore degenerate.

Lewis does not speculate as to the nature of subsequent compartmentalization events in *Drosophila*. However, a model built along similar lines would associate a unique control gene with each new compartment (see Figure 4–3). Thus, if gene *'e-ON'* determined segment 3, at the anterior–posterior compartmentalization event two new genes *g* and *h* would be activated, *g* specifying "anterior compartment of segment 3" and *h* specifying "posterior compartment of segment 3". In a cumulative system the continued activity of gene *e* would be required; in an epistatic system it would not. A subsequent dorso–ventral compartmentalization event would require the activity of four new genes in that segment, etc. Note that while ideographic systems require a greater total number of control genes than combinatorial systems, the information conveyed by the activity state of a particular gene is more specific, i.e. gene *'k-ON'* (Figure 4–3) specifies "the dorsal–posterior compartment of segment E". This property is important in the speculations which follow.

C. Mixed Combinatorial-Ideographic Systems

There is no *a priori* reason to expect real developmental systems to be organized along exclusively combinatorial or ideographic lines, and as has been noted, there is evidence in *Drosophila* for both types of system. Their spheres of control appear to correspond to evolutionarily primitive and more advanced stages, respectively, and I suggest there may be good reasons why this is so.

By a number of criteria segments appear to be discrete developmental units. Transplantation experiments with insect cuticle have led to the concept of segment boundaries as the limits of an anterior–posterior gradient which is serially repeated throughout the body (Locke, 1959). While recent work has shown that anterior–posterior position within segment may be specified by a co–ordinate system which is continuous

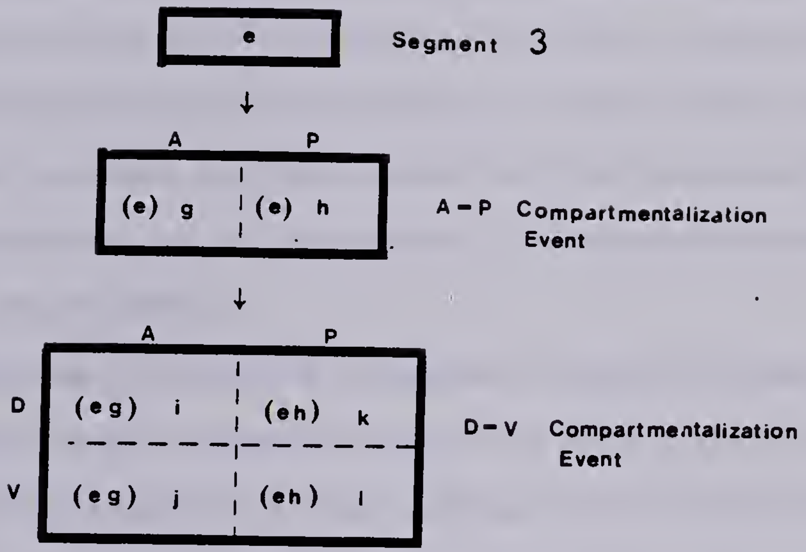


Figure 4–3. The genetic control of compartmentalization in a purely ideographic system. Genes which are ON are depicted within the compartments. At each compartmentalization event a unique gene is activated in each of the new compartments. In a cumulative system the continued activity of the genes in parentheses would be necessary, whereas in an epistatic system it would not.

across segmental boundaries (Wright and Lawrence, 1981), the clonal restrictions observed during normal development reveal that segments have different identities at a cellular level. These results imply that segments are differently determined in some sense. In Kauffman's combinatorial model a single set of control genes is responsible for both the establishment of segmental boundaries and the differing determined states of segments. Arguments presented above suggest that some degree of combinatorial control is expected, but two observations suggest that segmentation and determination are uncoupled in *Drosophila*: (i) the body of the hypothetical insect ancestor is thought to have been composed of a series of morphologically identical segments much like millipedes and centipedes today, and (ii) in *Drosophila*, mutants which disrupt segmentation define, in general, a different set of loci from mutants affecting determination. Segmentation and the determination of developmental level therefore appear to be different phenomena.

Segmentation may have arisen as a response to selection pressure for increased body size. It evidently resulted in reiterated units which were discrete, but whose developmental levels were identical, perhaps corresponding to the present "developmental sink". Throughout evolution there appears to have been selection for the diversification of segmental morphology and function, and segments have in general become modified one or a few at a time. I suggest that the most likely way for this to have occurred is by the superimposition of newly arisen ideographic control genes on the primitive combinatorial framework. Ideographic genes would have been selected for the efficiency with which they can interact with downstream genes. In a combinatorial system, each downstream gene which is segment- or compartment-specific must have a control region which can monitor the ON/OFF states of every gene involved in segment or compartment determination. An ideographic system would permit much simpler control of state-specific downstream genes since a single control gene would now be associated with each determined state. Thus, the process of segmental divergence may have proceeded by the evolution of control genes activated by positional information in single developmental units, and the subsequent gradual acquisition by these genes of control over sets of downstream genes which differentially modify the basal developmental pathway. In this view, the genes of the primitive combinatorial system may

currently be necessary for the establishment and maintenance of segments or compartments, and perhaps for the control of metabolic functions common to all segments, but to a large extent their roles in determination may have been superceded by other ideographic genes.

V. Interpretation of Control Gene Mutants

A. Definition of Terms

Before discussing mutations in control genes it will be helpful to define a few terms.

"Amorph, Hypomorph, Neomorph." Interpretation of a given mutation must take into account the nature of the genetic change involved (Muller, 1932). "Amorphic" and "hypomorphic" mutations cause a loss or lessening of wild-type gene activity. A mutation is considered to be amorphic if it has the same effect as a deletion of the locus, causing a total loss of function. Hypomorphic mutations retain some residual wild-type activity, such that addition of further doses of the mutant allele tends to restore the wild-type phenotype. "Neomorphic" mutations are those in which a function is acquired which is not present in the wild-type, and are defined operationally by the addition of extra doses of the wild-type allele. If this has no alleviating effect on the mutant phenotype, then the mutant is a neomorph. Neomorphs are therefore usually dominant. Mutants in which the normal function is expressed in a new embryological site would be classified as neomorphs.

"Pattern unit". In the discussions which follow, the general term "pattern unit" will be used when "segment" or "compartment" may be misleadingly specific. "Pattern unit" will refer to a set of structures differentiated in a discrete region controlled by a specific gene or set of genes. The pattern unit may be supra-segmental, segmental, or compartmental in nature.

"Domain, Range". There are two important characteristics associated with each homeotic transformation: (1) the pattern unit(s) which is transformed, and (2) the different pattern unit which appears in its stead. As we shall see, these characteristics provide insight into distinctly different aspects of the developmental program. I have suggested (Russell and Hayes, 1980) that a homeotic transformation is analogous to the mathematical transformation $f(x)=y$ where one set of elements, the domain, is transformed into a second set, the range. Thus, "the pattern unit which is transformed" will henceforth be referred to as the "domain" of the mutant, and "the different pattern unit which appears in its stead" as the "range" of the mutant.

"Autotypic, allotypic." A potentially confusing situation exists when one discusses a tissue type which occurs in two locations in the same fly due to a homeotic transformation, e.g. wing tissue in both the dorsal mesothorax and the eye due to the homeotic Mutation *Ophthalmoptera '(Opt-G)'* . In such cases I shall follow Ouweneel in referring to the normally located tissue as "autotypic" and the homeotically derived tissue as "allotypic". Thus, in *Ophthalmoptera*, "autotypic" wing structures are those found in the second thoracic segment, and "allotypic" wing structures are those which extrude from the eye.

B. Mutations in Control Genes

In various mutations of *Drosophila* it is observed that one pattern unit is replaced by another. In general, allotypic structures behave as if similar in every way to their autotypic counterparts. Mutations affecting particular structures also affect those structures when formed allotypically (see Ouweneel, 1976), and various parameters of development (Postlethwait and Schneiderman, 1971; Morata and Garcia-Bellido, 1976) have been found to be identical in allo- and autotypic structures. The simplest interpretation of these findings is that the same developmental pathway has been activated in both structures. This interpretation allows us to use the replacement patterns to make various deductions about the normal functions of the control genes involved. Deletions of control genes may have different properties than point mutants, and will be discussed separately in Chapter IX. In this chapter I will consider the replacement patterns expected of amorphic and neomorphic mutations in the genetic control systems described in section 4. The aims are two-fold: (i) to point out distinguishing characteristics by which the systems may be identified, and (ii) to make explicit the kinds of information which may be extracted from the domain and range of a mutant in a given system; mutants may provide insight into two levels of developmental organization, the activation of control genes and the control of determination by control gene interaction. The properties of pure systems will be considered first, followed by those expected of mixed systems.

Combinatorial Systems

The combinatorial systems proposed (Kauffman, 1973; Kauffman, Shymko, and Trabert, 1978; Garcia–Bellido, 1975) differ from ideographic systems, not only because they are non–degenerate but also because the same set of control genes is used to define the limits of pattern units and also to code for determined states within them. Although, as we have seen, this cannot generally be true for segmentation, it is a central feature of the selector gene model for compartmentalization. In this case, mutations in control genes may affect the number of units as well as their determined states.

Let us examine the effects of amorphic mutations in combinatorial systems. A consequence of non–degeneracy is that the gene is active in determination whenever it is ON. An amorphic mutation effectively results in the gene being OFF in all locations, and therefore provides information about the control of activation of the gene. The domain of the mutant -- the set of pattern units lost –reveals the regions where the gene is ON in the wild–type; the range of the mutant corresponds to the pattern units where the gene is OFF in the wild–type. Figure 5–1 shows the effects of amorphic mutants on a linear array of eight combinatorially defined pattern units. A critical assumption is that the activation of a given gene is independent of that of other genes. There are three aspects worthy of note. First, the members of a pair of domain–range pattern units are identical for the activity states of all control loci other than that which is mutant. Second, there are four different pattern units in both the domain and range; in general, transformations in combinatorial systems are "many to many". Third, the creation of a duplicate set of range pattern units in the mutant will result in the fusion of adjacent pattern units only when these pairs of units are contiguous.

Amorphs result from total loss of function, and may therefore be interpreted unambiguously. Neomorphic homeotics, however, are thought to result from activation of control genes in locations where they are usually silent. Their phenotypes must be interpreted with caution as one has no assurance that the mutation will be activated uniformly throughout the entire system. By reasoning analogous to that used in the discussion of amorphs, one may interpret regions which are transformed as ones in which the gene is OFF in the wild–type, but without further evidence one cannot equate untransformed regions with ones in which the gene is normally ON. Untransformed

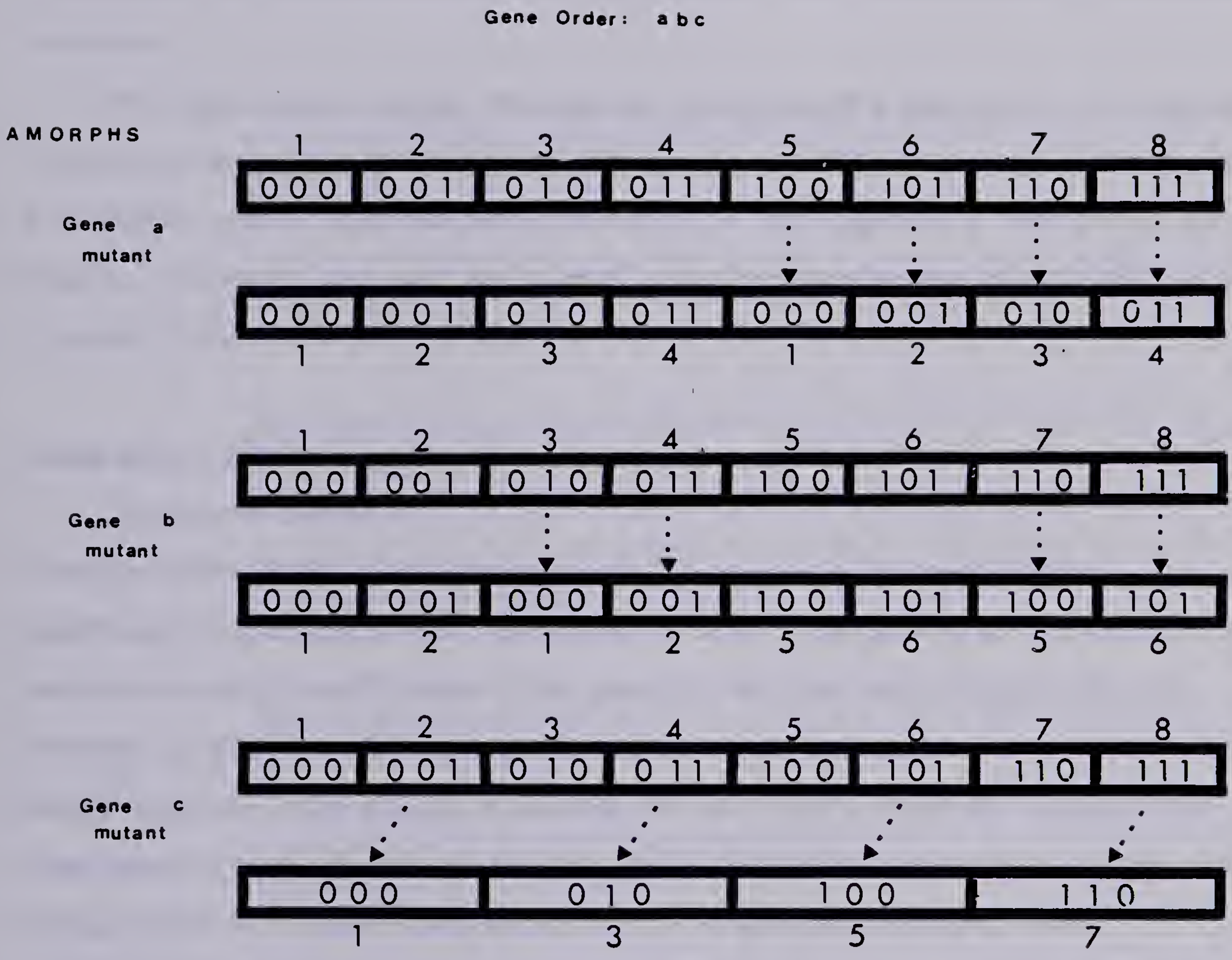


Figure 5–1. The expected effects of amorphic mutations in control genes of a combinatorial system. In each mutant case the wildtype array of segments is written above and the mutant array below. The arrows indicate the transformations caused by the various mutants. Segmental identities are written above for the wildtype arrays and below for mutant arrays. See text for further discussion.

regions may also represent regions in which the gene is OFF in both the wild-type and the mutant because the mutation does not result in a ubiquitous activation of the control gene. In specific cases a resolution of this dilemma may be possible based upon the interactions of a neomorph with another mutant, and this will be discussed in Chapter VIII. Throughout this section, I will assume that the distinction between the OFF and ON states of a neomorphic gene can be made, and I will discuss the effects expected of ubiquitous neomorphs.

In a combinatorial system, the expected phenotype of a neomorph is the opposite of that of an amorph for the same gene. The gene is now ON in all regions, so the domain is the pattern units in which the gene is normally OFF, etc. (Figure 5-2). Note that as with amorphs, neomorphs cause a "many to many" transformation and may result in the fusion of pattern units.

Ideographic Systems

Cumulative Systems

In the cumulative model, developmental levels are ordered in the sense that the determination of any given level requires the ON state of the gene which corresponds to that level as well as the ON states of the genes for all lower levels. Figure 4-2a is an illustration of such a model showing the gene state-sets associated with each determined state. In Figure 5-3, the effects of amorphic and neomorphic mutations in each of the three genes of such a system are depicted. Because the code is degenerate, not all code changes result in changes in determined state. Of interest are the cases where transformations between states are observed. Amorphic mutations will cause ON states of genes to become OFF, or 1 to become 0 in the notation of the figure. These mutations will cause the transformations shown by the leftward pointing arrows. As can be seen in the table, the number of pattern units in the range is always one, but the number of units in the domain may vary from many to one depending upon the gene involved; the expectations are thus quite distinct from those of a combinatorial system. One may conclude from amorphs that the gene is normally ON in the pattern units of the domain (but not necessarily exclusively there) and OFF in those of the range, and that the range pattern unit is a lower level of development than the domain pattern unit.

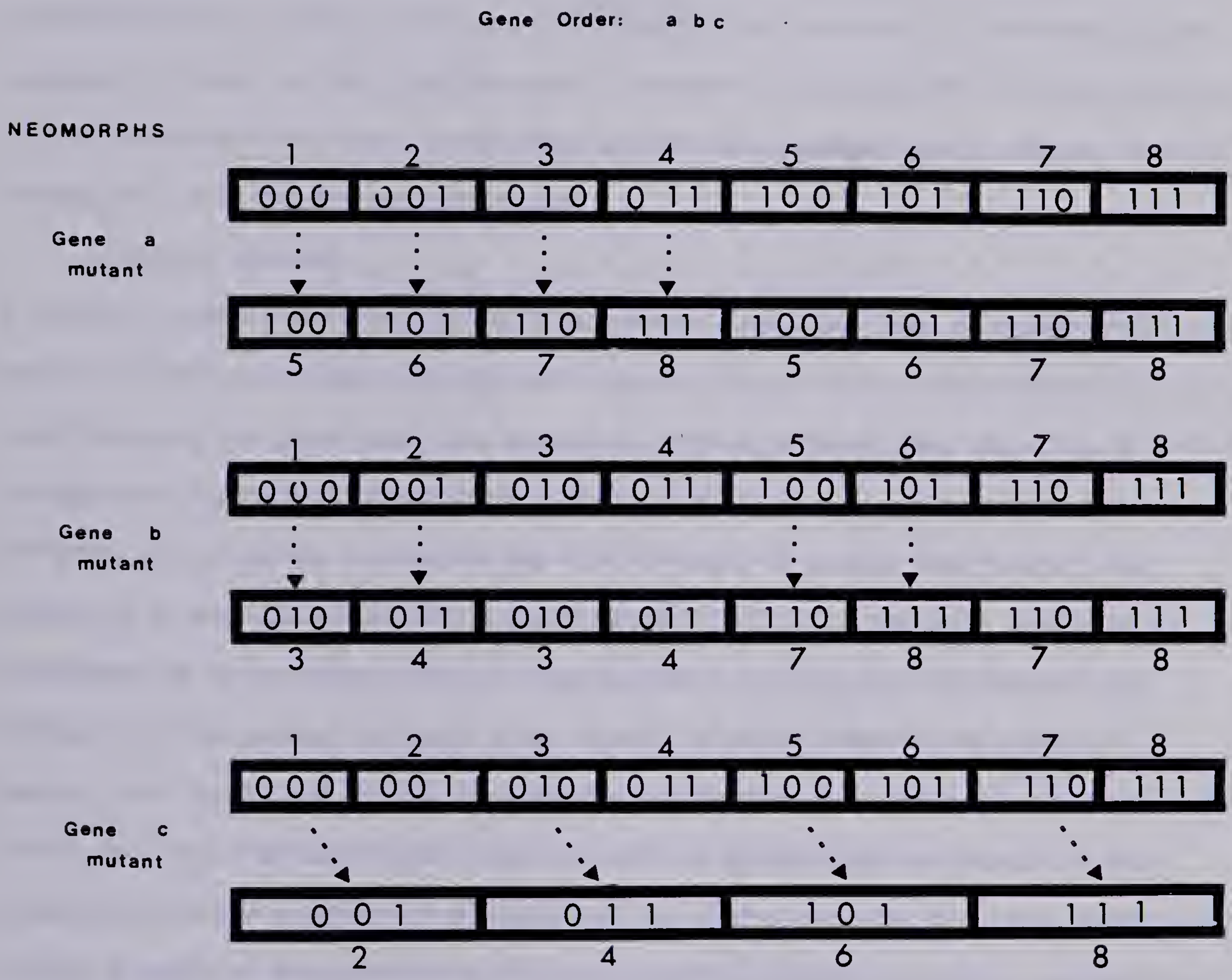


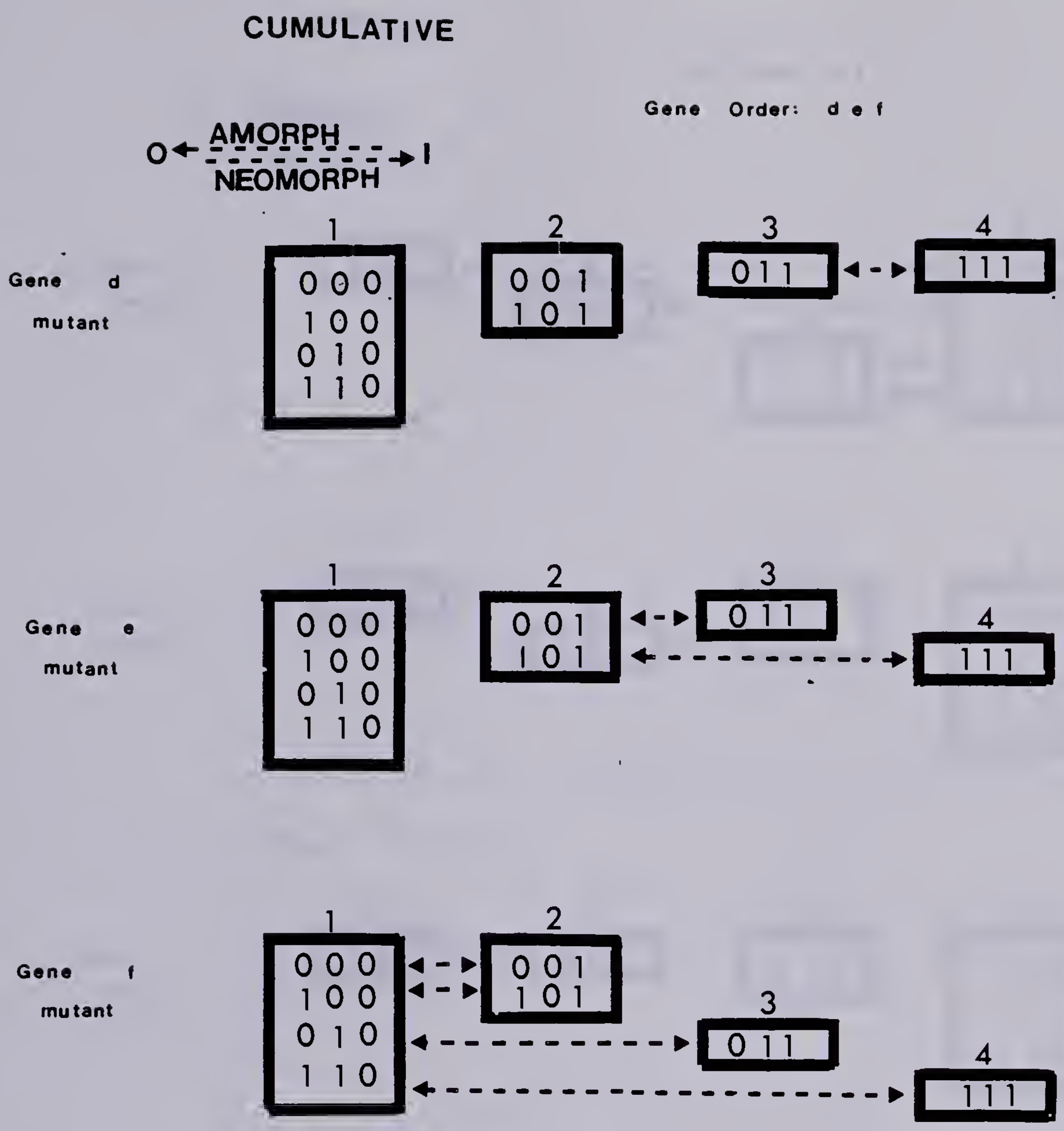
Figure 5-2. The expected effects of neomorphic mutations in control genes of a combinatorial system (as in Fig. 5-1).

The transformations expected of neomorphs are the opposite of those expected of amorphs and are shown by the rightward facing arrows in Figure 5–3. As the table shows, the transformations are all "one to one" with a unique pattern unit in the domain and various possible unique pattern units in the range, depending upon the underlying states of the other control genes involved. Note that even with a ubiquitous neomorph one expects only a single pattern unit in the range. The conclusions to be made are the opposite of those with amorphs: the gene in question is normally OFF in the pattern units of the domain and ON in that of the range, and the range pattern unit is a higher level of development than the domain pattern unit.

Epistatic systems

In epistatic systems each control locus determines a particular level of development, and there is an ordered epistasis among control genes. Figure 4–2b is an illustration of such a model showing the gene state–sets associated with each determined state. Figure 5–4 is analogous to Figure 5–3, showing the effects of amorphs and neomorphs in the different control genes. In amorphs the transformation is always "one to one". The domain of an amorph reveals only a single region of action of the gene, and gives no information as to its activity states in other pattern units where its effects may be masked by other genes. The range of an amorph, however, reveals the activity of a second, wild–type control gene whose effect is normally overridden in the domain in the wild–type. Thus, if enough transformations with the same range are found, one may deduce the complete pattern of activation of the control gene for the range. Additionally, through a series of amorphs in the different epistatic control loci one may deduce the order of epistasis among them. If a given transformation is not to the next lower level in the order, any intervening control genes must be OFF in that domain (i.e. in Figure 5–4, if 4 is transformed to 1, then the control genes for 2 and 3 must be OFF, if 4 is transformed to 2, then the control gene for 3 must be OFF).

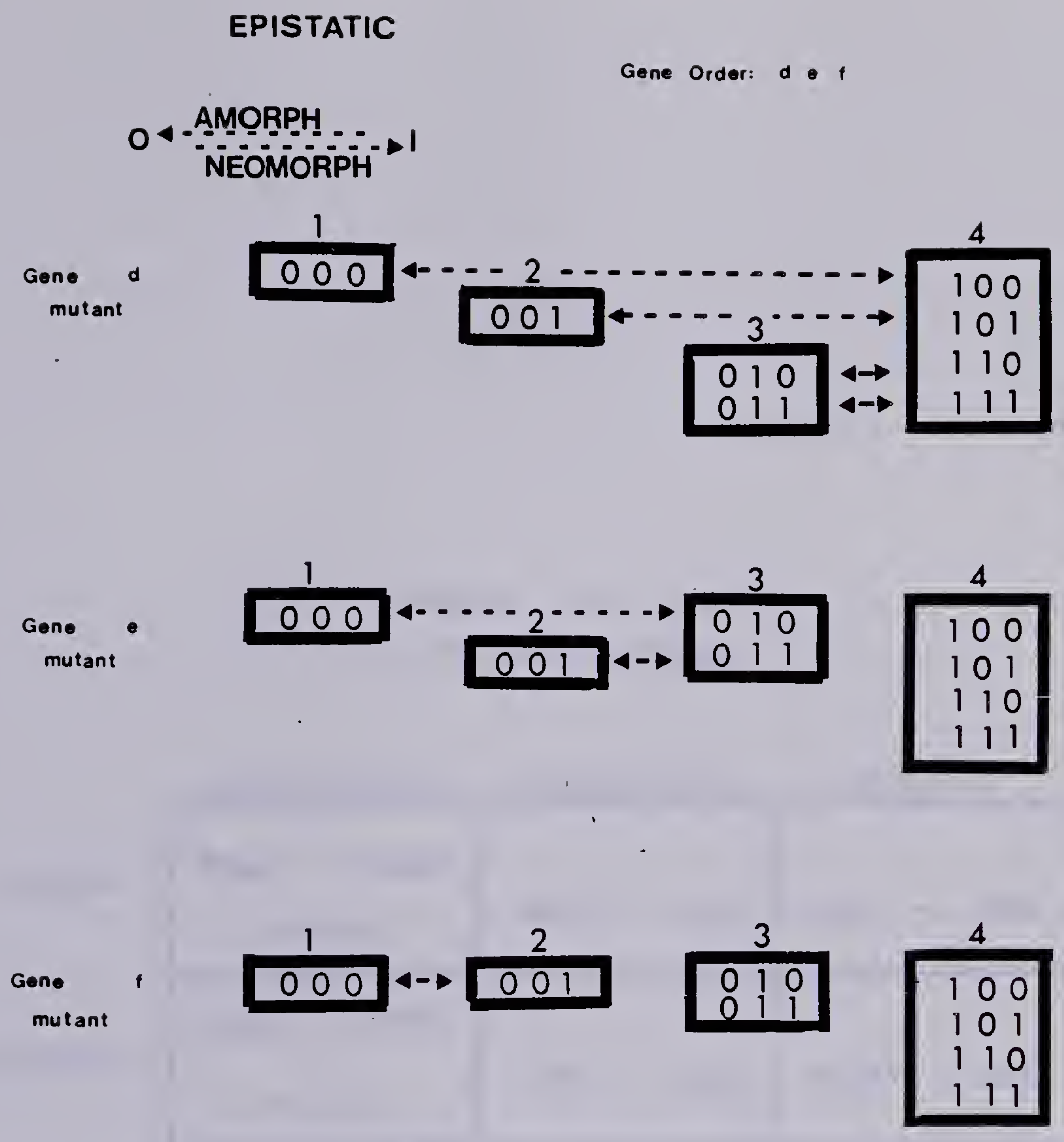
The expected phenotypes of ubiquitous neomorphic mutations are quite simple: (see table in Figure 5–4) all pattern units lower in the epistatic order will be transformed to the pattern unit of the gene in question; structures higher in the order will be unaffected. Note that here as in cumulative systems one expects only a single pattern unit in the range of a ubiquitous neomorph. Figure 5–5 summarizes the properties expected



	Gene	Domain	⟶	Range
Amorph	d	4	→	3
	e	4 and 3	→	2
	f	4 , 3 and 2	→	1
Neomorph	d	3	→	4
	e	2	→	4 or 3
	f	1	→	4 , 3 or 2

Figure 5–3. The expected effects of amorphic and neomorphic mutations in genes of a cumulative ideographic system. The boxes enclose the wildtype and any degenerate codons for the segment as numbered at the top. The effects of an amorphic mutation in a given gene may be seen to be the leftward pointing arrows, i.e., an amorph in gene d will transform segment 4 (1 1 1) into segment 3 (0 1 1). The effects of neomorphs may be seen to be the opposite, shown by the rightward pointing arrows, i.e., a neomorphic mutation in gene d will transform segment 3 (0 1 1) to segment 4 (1 1 1) but will leave segments 1 and 2 untransformed due to the degeneracy of the code. The effects are summarized in the table at the bottom.

[illegible]



	Gene	Domain ⟶	Range
Amorph	d	4 →	1, 2 or 3
	e	3 →	1 or 2
	f	2 →	1
Neomorph	d	1, 2 and 3 →	4
	e	1 and 2 →	3
	f	1 →	2

Figure 5–4. The effects of amorphic and neomorphic mutations in genes of an epistatic ideographic system (as in Fig. 5–3).

DOMAIN → RANGE
TRANSFORMATIONS

	COMBINATORIAL	CUMULATIVE	EPISTATIC
AMORPH	MANY → MANY (FUSION)	MANY → ONE	ONE → ONE
NEOMORPH	MANY → MANY (FUSION)	ONE → ONE	MANY → ONE

Figure 5–5. A general summary of the effects of amorphs and neomorphs in the three systems.

of mutant transformations in the three models. Mutants in combinatorial systems may be distinguished from those in ideographic systems by the fact that they would all contain multiple pattern units in the range, and some in addition would cause the fusion of adjacent pattern units. In ideographic systems the range would consist of a single pattern unit. The two ideographic systems may be distinguished from each other only if one knows the nature of the mutation, since amorphs and neomorphs have reciprocal properties in the two systems.

Mixed Systems

In real developmental systems the final determined state is most likely encoded by a mixture of combinatorial and ideographic components. The effect of mixed systems in general is to decrease the degree of resolution of the criteria in Figure 5–5. As ideographic genes require the functioning of an underlying system to establish segments, any mutation which results in the fusion of segments will still be fully expressive in mixed systems. However, mutations which in a pure combinatorial system would result only in the transformation of segments (see Figure 5–1) will have the number of units in their domain reduced in mixed systems where ideographic genes now control determination. Thus, the "many to many" transformation of a mutant in a combinatorial system may be reduced in a mixed system to "few to few", which is still recognizable as a combinatorial pattern, or ultimately to "one to one", at which point the gene is functionally ideographic.

I turn now to a discussion of particular homeotic mutants in *Drosophila*.

VI. Description of Homeotic Mutants

Homeosis is a general term which refers to the replacement of one body structure by a duplicate of another. Many treatments may result in such replacements without genetically altering the cells involved, e.g. cell proliferation in *in vivo* culture (transdetermination), or exposure of embryos to heat shock, ether vapor or a variety of other agents (see Ouweneel, 1976 for review). Here I will discuss only those examples of homeosis which result from mutations, as they identify potential control loci.

A. Caveats

Mutations at many different loci in *Drosophila melanogaster* are known to cause homeosis. While this behavior is expected of mutants in control loci it is clear that not all such mutants identify genes directly involved in the control of development. One class of mutations causing what Ouweneel (1976) refers to as "intra-disc homeosis" almost certainly does not define control loci. Several mutants isolated as temperature-sensitive cell lethals replace structures with mirror image duplications of neighboring structures following pulses of the restrictive temperature during development. However, the resulting phenotypes mimic those seen when wild-type discs are allowed to regulate after surgical operations (Bryant, 1971); it has been suggested that conditional cell lethal mutations cause localized cell death in discs resulting in disc fragments which subsequently duplicate (Russell, 1974). Cell lethals causing intra-disc homeosis are thus not believed to identify loci integrally involved in determination.

Less easily recognized are mutations which result in transformations due to other unidentified secondary effects, but this class must surely be a common one. *Ultrabithorax-like (Ubl)* is an example of this type (Morton and Lefevre, 1981). Originally isolated because in heterozygous condition it causes a partial transformation of haltere to wing, *Ubl* was subsequently found to be a mutation at a locus coding for a subunit of RNA polymerase (Greenleaf *et al.*, 1980). Its homeotic effect may thus be the result of a more generalized effect on transcription. Unfortunately *Ubl* is the only homeotic mutation whose wild-type function has been characterized at the molecular level, and in general the weeding out of mutants which cause homeosis due to secondary

effects is not a straightforward process.

By what criteria then does one identify loci whose primary function is to control access to particular developmental pathways? There are four characteristics of such control genes which may aid in their identification from mutant phenotypes:

(1) Genes which control access to specific developmental pathways at blastoderm are thought to be activated in response to a positional information gradient influenced by gene activity in the oocyte (Sander, 1975b). Mutations in genes which respond to the gradient may be distinguished from those which establish or modify the gradient by the fact that the latter class would show maternal effects (e.g. *bicaudal, dorsal*) while control gene mutants would have only zygotic effects.

(2) Transformations in secondary fields may similarly result from mutants in genes which establish positional information or in control genes which respond to positional information. These two classes of mutant may be distinguished to some extent by the criterion of cellular autonomy. Autonomous behavior in clones would be typical of control genes with positive feedback memory systems. Non–autonomy would be expected of two classes of mutant: (i) mutants in genes which establish positional information would behave non–autonomously if, as seems likely, surrounding wild–type cells were sufficient to generate field properties; and (ii) mutations in control genes which are only necessary for the initial activation of a given pathway (see Holliday and Pugh, 1975) would be non–autonomous in clones because the memory function would remain intact at a separate locus.

(3) As measured by temperature–sensitivity, clonal analysis, or other methods, the timing of gene action should correspond to the period when the control gene would be expected to act, on the basis of the developmental decision it controls.

(4) The effects of a mutant should be limited to a pattern unit or units, as defined by some other criterion. The domain of a hypomorph and the range of a partial neomorph may be less than a complete pattern unit, but no mutant effects should transgress unit boundaries. *Ubl* fails to meet criteria (1) and (4), and therefore would not have been considered to define a control locus even were its biochemical nature unknown.

The remainder of this thesis will consider a set of mutants which meet these four criteria and appear to define control loci involved in the determination of the meso– and

metathoraces and the first abdominal segment. In the following section I describe the wild–type phenotype of these segments with particular reference to compartment specific markers. I then consider the characteristics of mutants with respect to these markers and to the criteria above.

B. The Wild-type Phenotypes

The adult meso– and metathoraces are each derived from two imaginal discs, a dorsal disc giving rise to the wing and mesonotum (which I shall call the "wing disc") or a haltere and metanotum ("haltere disc"), and a ventral disc giving rise to the mesothoracic leg or metathoracic leg.

Clonal analysis reveals that at the cellular blastoderm stage both the meso– and metathoracic primordia are divided into anterior and posterior compartments, but the dorsal and ventral discs of the same segment are not clonally distinct (Steiner, 1976; Wieschaus and Gehring, 1976). For a complete description of the wing, the reader is referred to Bryant (1975). Figure 6–1 shows the wing with a number of features of particular interest. Each wing–blade cell secretes a single trichome. The size and spacing of trichomes is fairly uniform throughout the wing blade, and is characteristic of the wing. The anterio–posterior compartment boundary (A–P boundary) defines a straight line running anterior to vein IV. The dorso–ventral compartment boundary (D–V boundary) runs along the margin of the wing. At the D–V boundary in the anterior compartment a pattern of bristles is seen called the "triple row" which consists of a large median row of bristles and two rows of smaller bristles, the dorsal row being more widely spaced than the ventral. The D–V boundary separates medial from ventral row elements. Proximal to the triple row elements is the costa, with its characteristic socketed bristles. At the D–V boundary in the posterior compartment a double row of socketless hairs may be found, called the "posterior row". Proximal to the posterior row is the alar lobe, with its characteristic long, unsocketed hairs.

The mesonotum comprises most of the dorsal surface of the thoracic region. The anterior compartment contains thirteen recognizable macrochaetae and around one hundred microchaetae per hemithorax (Morata and Kerridge, 1980). The posterior

Figure 6-1. The wildtype wing. Note the socketed bristles of the triple row (TR) and costa (Co) in the anterior and the socketless hairs of the posterior row (PR) and alula (AL).

Figure 6-2. The wildtype haltere.

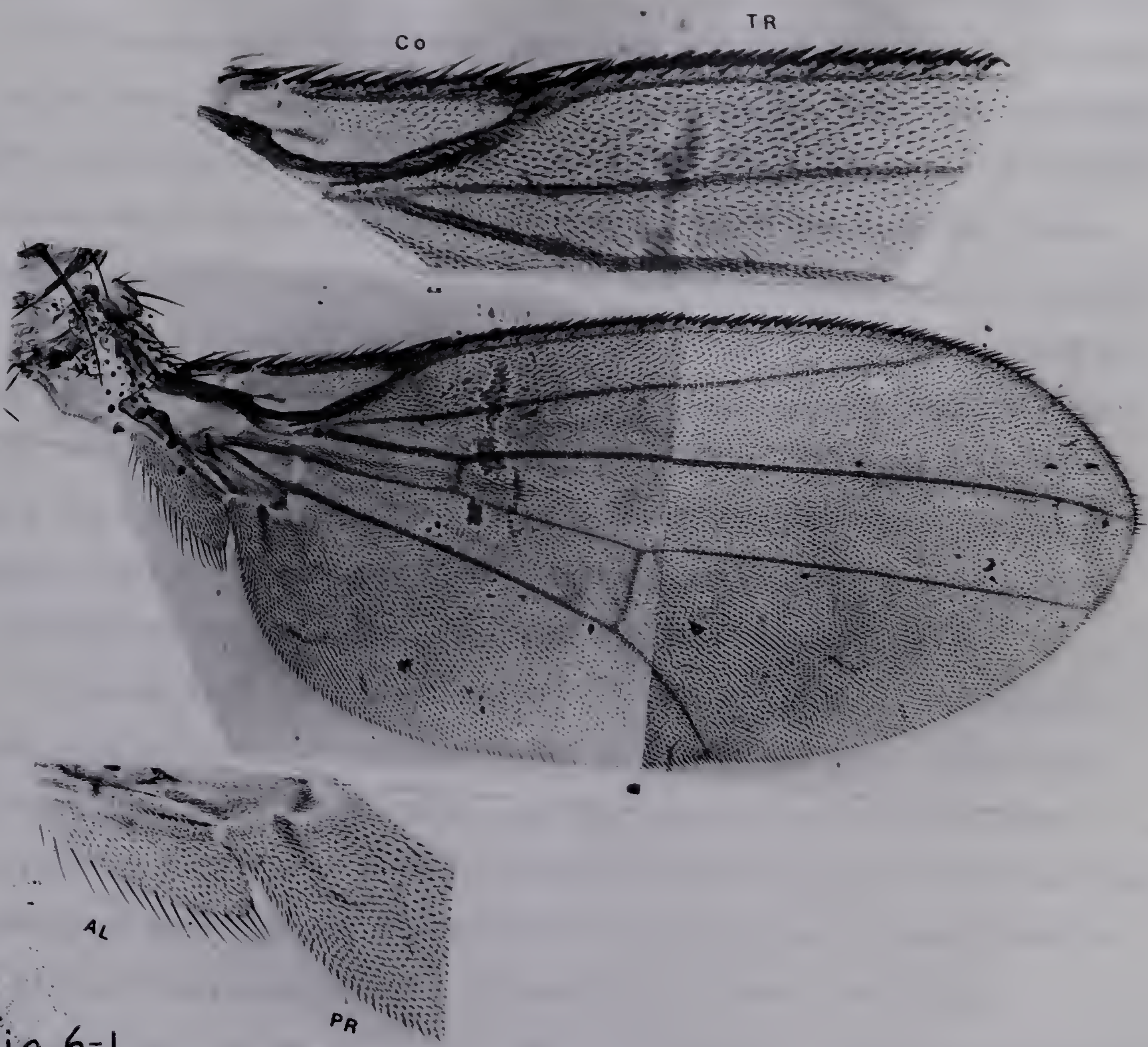


Fig. 6-1

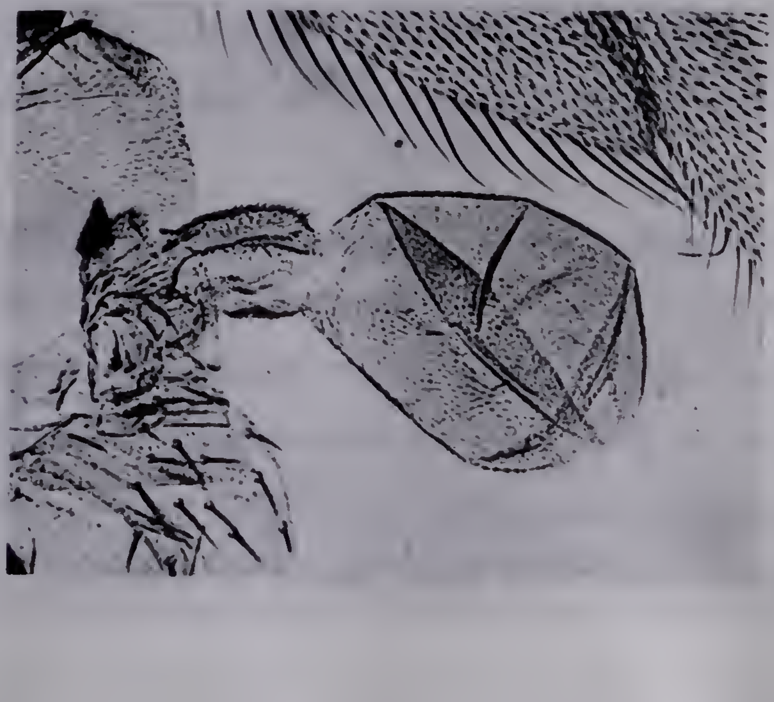


Fig. 6-2

compartment is restricted to the post-notum, a small strip of cuticle bearing no bristles.

The derivatives of the wild-type haltere disc have been described by Ouweneel and van der Meer (1973) (see Figure 6-2). The haltere sclerite is quite small and laterally located, and carries the metathoracic spiracle, a small bristle group and a pair of papillae. The haltere proper may be divided into three regions, a proximal scabellum, a medial pedicellum, and a distal capitellum. The scabellum and the pedicellum have a recognizable A-P asymmetry and bear distinct groups of sensilla campaniformia on their dorsal and ventral surfaces. The capitellum is a symmetrical structure bearing what Ouweneel and van der Meer describe as two or three groups of sensilla trichodea but which in the material I have examined always fall into two distinct groups, a compact clump generally numbering from 3 – 8 located proximally on the dorsal surface and a curving line of 10-18 extending distally and anteriorly on the ventral surface (Figure 6-3).

To locate these markers with respect to the A-P boundary in the haltere, the *Minute*$^{+}$ *multiple wing hairs* (*M*$^{+}$*mwh*) clones generated by E. Steiner (1976) were scored. As described in Steiner (1976), eggs and larvae of the of the genotype *y; Dp(1:3)sc*^{4}*, y*$^{+}$ *M(3)*^{55}*/mwh jv* were irradiated at times ranging from 3±0.5 hours until 132±12 hours after egglaying; *M*$^{+}$*mwh* clones in the haltere were scored by plotting their contours on a standard drawing of the haltere. All clones were scored independently by E. Steiner and myself; in spite of the paucity of markers, we found only small discrepancies between the outlines of a few clones with the majority of cases being in complete agreement. The results are shown in Figure 6-4. A line could be drawn which no clone crossed, possibly delimiting a compartment boundary; it is reasonable to assume (Steiner, loc. cit.) that this corresponds to the anterior and posterior boundary, the anterior compartment being defined by the inclusion of anterior structures in the pedicellum.

The proximal clump of sensilla was generally found in the posterior compartment on the dorsal surface, but in a few cases it straddled the compartment border, suggesting some degree of indeterminacy in its location. This phenomenon has been described before in other systems (Morata and Lawrence, 1979). As the number of sensilla also varies from fly to fly it seems likely that a region with the capacity to form sensilla straddles the A-P compartment border, being for the most part in the posterior

Figure 6–3. The dorsal and ventral surfaces of the haltere, showing the location of the sensilla groups.

Figure 6–4. The outlines of M^{+}*mwh* clones on the haltere. The dotted lines separate anterior (A) and posterior (P) compartments.

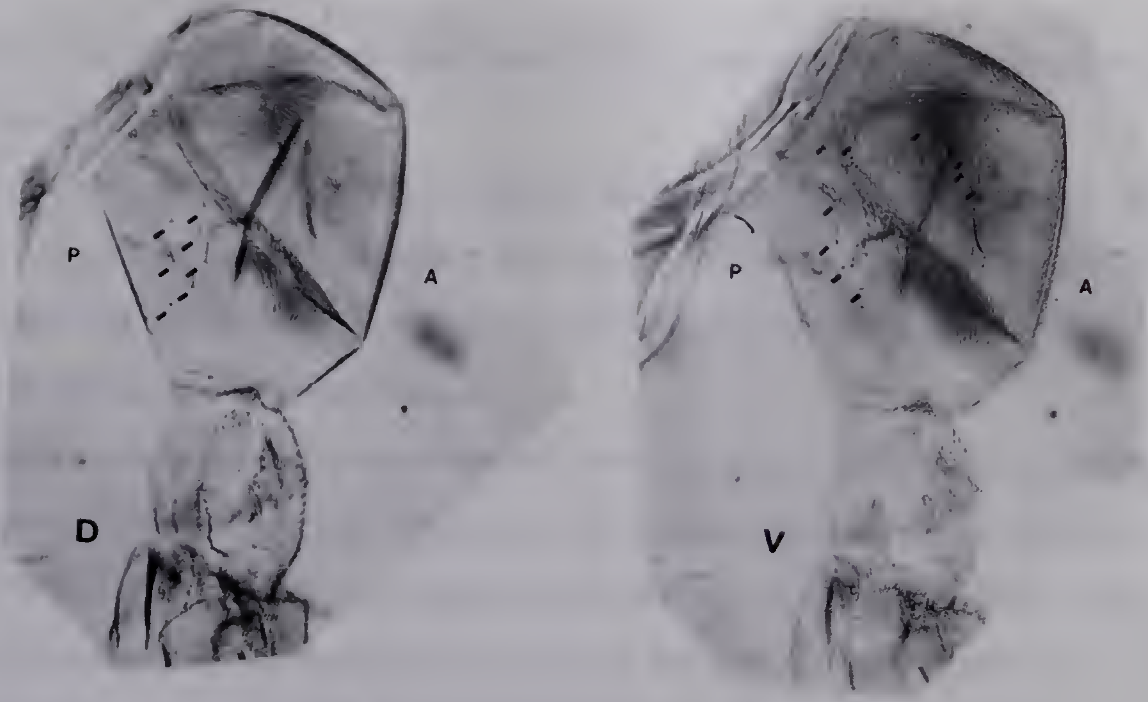


Fig. 6-3

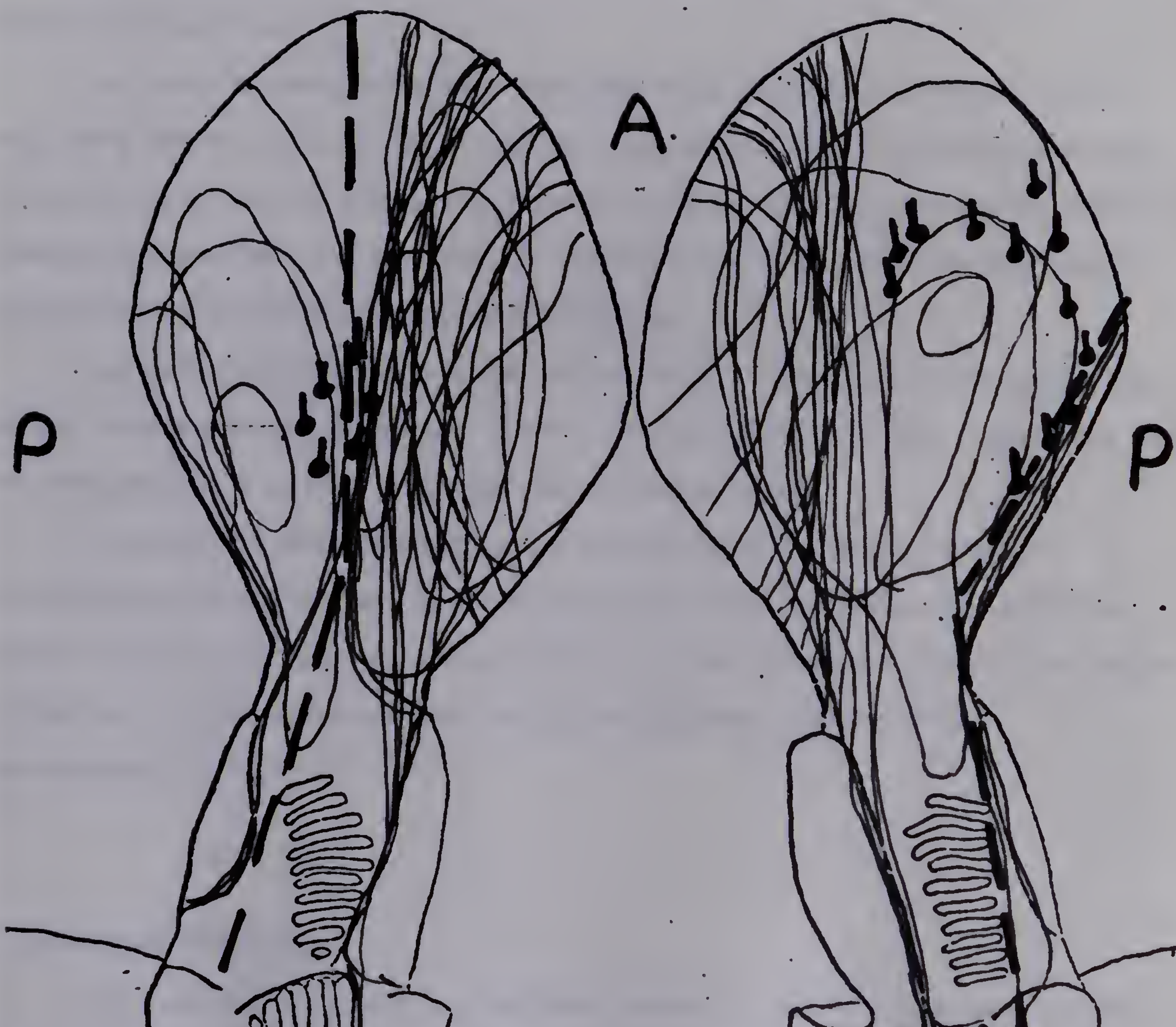


Fig 6-4

compartment, and that local interactions determine the number and precise location of individual sensilla in this region.

The curving line of sensilla was found to extend distally along the anterior side of the ventral A–P compartment border and then to curve anteriorly. In a few cases one or two of the most proximal sensilla were found in the posterior compartment, again suggesting some degree of indeterminacy.

These results are in general agreement with the data of Morata and Garcia–Bellido (1976) as depicted diagramatically in Capdevila and Garcia–Bellido (1974). The sole difference is that in the results presented above the majority of the proximo–distal stretch of the curving line of sensilla was found on the anterior side of the A–P boundary, whereas Garcia–Bellido and Capdevila show it to be mostly within the posterior compartment.

The size and spacing of haltere trichomes differ markedly from those of the wing, being smaller and more densely packed. They are also characteristically oriented in a proximo–distal direction except in a distinctive area of the most distal region where the orientation is confused. This area may correspond to the most distal point specified by the positional information system of the haltere disc.

The ventral discs give rise to the five segments of the legs and several proximal thoracic sclerites. Figures 6–5 and 6–6 show the second and third legs, respectively, and the markers specific to each. A summary is provided in Table I.

The adult first abdominal segment is represented in the larva by the three histoblast nests typical of other abdominal segments. After pupariation, however, the ventral histoblast nest does not develop and the adult structures are limited to the tergite formed by the anterior and posterior dorsal histoblast nests (Madhavan and Schneiderman, 1977).

C. The Mutant Phenotypes

The *engrailed* locus (*en*). Two different classes of mutation have been isolated at the *en* locus, embryonic lethals which result in abnormalities in segmentation (Kornberg, 1981) and the original allele *en*^{1} (Ecker, 1929) which is homozygous viable and causes

Figure 6-5. The markers characteristic of the wildtype second leg. (see Table 1 for a summary of the markers)

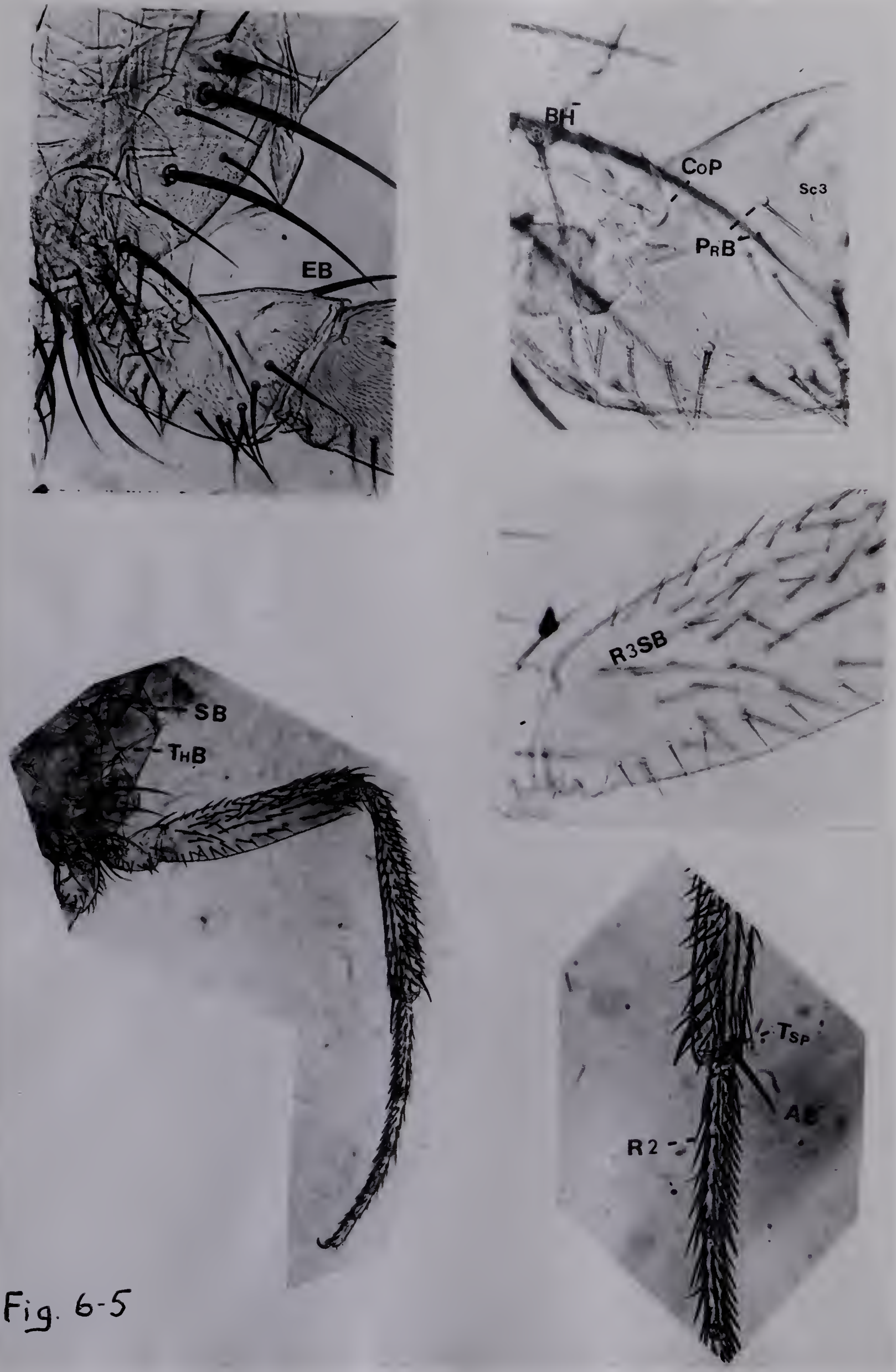


Fig. 6-5

Figure 6-6. The markers characteristic of the wildtype third leg. (see Table 1 for a summary of the markers)

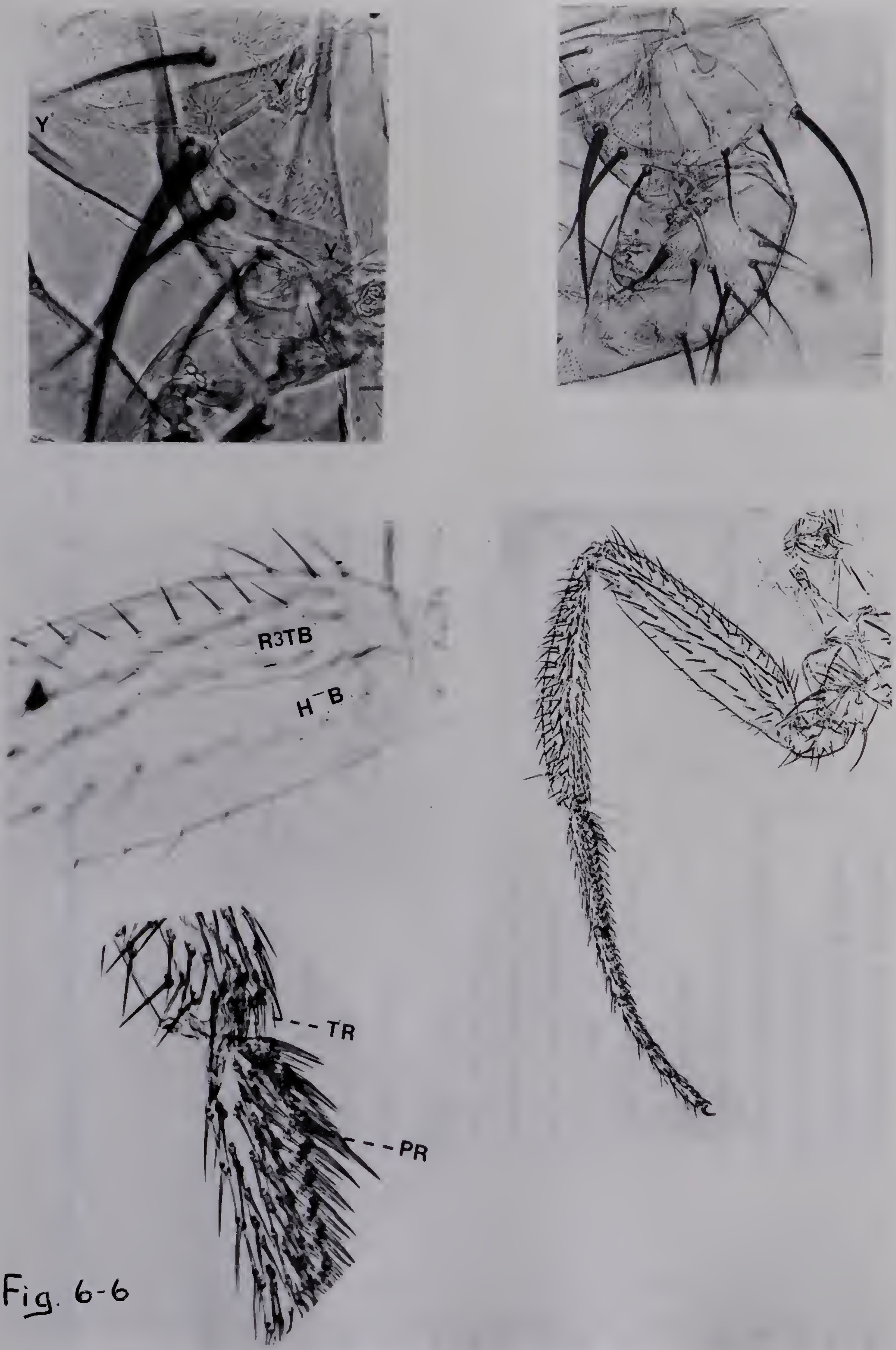


Fig. 6-6

Table 1. The location and compartment specificities of markers in the second and third legs.

			COMPARTMENT SPECIFICITY			
MARKER	DESCRIPTION	LOCATION	AMS	PMS	AMT	PMT
SB	Sternopleural Bristles	Leg Sclerite	X			
ThB	Thoracic Bristles	Leg Sclerite	X			
EB	Edge Bristle	Trochanter	X			
AB	Apical Bristle	Tibia	X			
CoP	Coxal Process	Coxa		X		
BH$^-$	Hairy Island Bristle	Coxa		X		
PrB	Bristles proximal to sc3	Trochanter		X		
R3SB	Row 3, Small Bristles	Femur		X		
Tsp	Tibial Spurs	Tibia		X		
R2	Single Row 2 of Bristles	Tarsus		X		
Y	Y-shaped Apodeme	Coxa				X
iD	Bristle distal to sc3	Trochanter				X
H$^-$B	Small bristles in proximal femur	Femur				X
R3TB	Row 3 Tiny Bristles	Femur				X
TR	Transverse Row of Bristles	Tibia				X
PR	Transverse Row of Bristles	Tarsus				X

pattern abnormalities within segments. In *en*–lethals, the defect appears to be in the maintenance of segment borders rather than in the original definition of segments, for segments are observed to form initially and subsequently to become fused. The patterns of segmental disruption appear to be allele–specific, and range from the fusion of varied numbers of different segments to the fusion of specific pairs of segments throughout the embryo, in either of two pairwise registers (Kornberg, 1981).

The en^1 mutation causes a different spectrum of effects from those seen in *en*–lethals: it causes a transformation of posterior compartment structures to anterior compartment structures in at least two segments, and also abolishes the A–P compartment boundary in at least one of the two, but it does not affect the integrity of the segments themselves, as do *en*–lethals. It has been suggested that the embryonic segmental fusions found in *en*–lethals also result from the loss of A–P distinctions within segments (Kornberg, 1981), but it is unclear as yet why different allele–specific patterns of fusion would be generated if this were the case. The possibility thus exists that *engrailed* is a complex locus; the phenotype of en^1 will therefore be considered without reference to *en*–lethals.

In the first leg, en^1 causes an autonomous transformation of certain posterior structures to the corresponding anterior structures symmetric about the A–P boundary (Tokunaga, 1961) (see Figure 6–7). A similar phenotype occurs in the wing (Garcia–Bellido and Santamaria, 1972) (see Figure 6–8) where it has in addition been observed that the transformation of posterior to anterior is accompanied by the loss of the A–P border. It was found that the transformation as well as the behavior of clones in the wing could be explained by assuming that *en* is normally ON in the posterior compartment and OFF in the anterior, and that the difference between the ON and OFF states was responsible for the compartment border (Lawrence and Morata, 1976).

The en^1 mutation thus exhibits the two diagnostic characteristics of control genes in combinatorial systems -- multiple pattern units in the range and the fusion of adjacent pattern units (see Figure 5–5). However, if *Drosophila* is a mixed combinatorial–ideographic system one would not expect that similar transformation would occur in the posterior compartments of all segments. In the second and third legs, en^1 causes the appearance of extra bristles in the posterior compartment of the tarsi, and

Figure 6–7. Wild–type and *en*^{1} first legs. Note the mirror symmetric arrangement of the sex–combs in the *en*^{1} leg.

Figure 6–8. The *en* wing. Note the socketed bristles on the alula and triple row bristles along the posterior margin (cf. Figure 6–1).

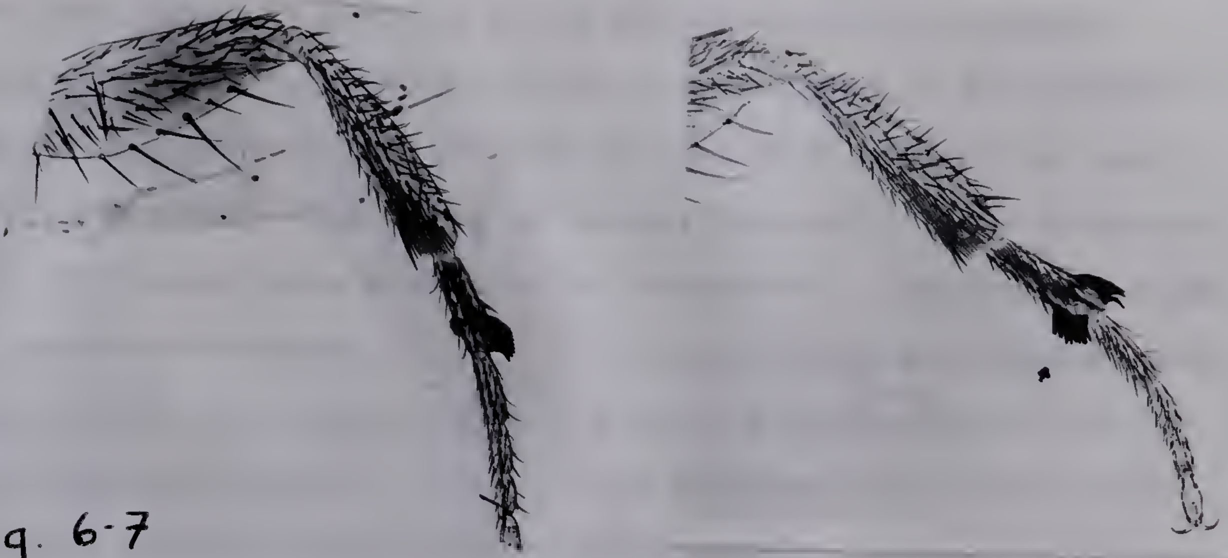


Fig. 6-7

Fig. 6-8

this has been interpreted as evidence of a posterior to anterior transformation (Lawrence *et al.*, 1979). However, in en^1 second and third legs it is also common for the posterior terminal claw to be absent altogether or else duplicated (S. Eberlein, personal communication). As the two terminal claws are symmetric about the A–P boundary, deficient or supernumerary claws would not be expected of a posterior to anterior homeotic transformation; they may be explained as the result of regulative responses to cell death in different regions of the posterior compartment, as may the extra bristles mentioned above. In the haltere disc it has been observed that the en^1 mutation causes changes in the activation pattern of specific enzymes as revealed by histochemical staining procedures (Sprey *et al.*, 1981). It has been claimed that en^1 also causes a duplication of anterior markers in the haltere (Garcia–Bellido, Lawrence, and Morata 1979) In my material I find the morphology of the haltere unchanged by en^1. I examined a number of halteres from four en^+ strains and three strains either homozygous for en^1 or heterozygous for en^1 and two deficiencies of the *en* region isolated by M. Russell which give an *en* phenotype in the wing when heterozygous with en^1 (S. Eberlein, personal communication). The gross morphology of *en* halteres was in no way different from the wild–type strains as was also true of the locations and number of sensilla on the capitellum (Table 2, lines 1–7). Thus, it appears that in the metathorax and the second leg the engrailed product still functions, since patterns of enzyme activity are influenced by the mutant, but the absence of a homeotic effect in the metathorax suggests that its role in specifying particular developmental pathways may be segment specific.

The Bithorax Complex. The Bithorax Complex is the name given to a genetically small region on the third chromosome which contains a number of loci involved in determination (see Figure 6–9). Extensively studied by E.B. Lewis (1951, 1954, 1955, 1963, 1964, 1967, 1968, 1978), the complex yields at least eight classes of mutants which cause transformations between various thoracic and abdominal segments. The proximal region from *bx* to *pbx* has been most extensively studied thus far, and will be discussed in this thesis. The mutant phenotypes in the dorsal discs have been well described except with respect to the capitellar sensilla which I discuss here for the first time. The ventral transformations are less well known, and will be described with reference to the compartment specific members listed in Table 1. Mutations causing loss

Table 2. The number of sensilla on dorsal and ventral surfaces in various genotypes. *l(1)ts726* is a temperature–sensitive cell lethal mutation (Russell, 1974). Df(2) *en*^{21} and Df(2) *en*^{30} are deficiencies in the *en* region which fail to complement *en*^{1} (Russell and Eberlein, 1979). Df(3)P9 is a deficiency for the entire Bithorax Complex. T(2:3) *Hm* and Df(3)P9 were kindly supplied by Prof. E.B. Lewis. Other genotypes are described in Lindsley and Grell, 1968, and were obtaind from the Bowling Green or California Institute of Technology *Drosophila* stock centers.

GENOTYPE		# SENSILLA ON DORSAL SURFACE (CLUMP)			# SENSILLA ON VENTRAL SURFACE (LINE)		
1.	*pwn en/M(2)c*^{33} *;mwh jv/+*	6.06	±	0.37	13.20	±	0.57
2.	*y v f l(1)ts*^{726}*/+*	5.36	±	0.40	11.82	±	0.47
3.	*mwh red e*	6.56	±	0.29	11.85	±	0.37
4.	*l(3)4*	5.30	±	0.34	11.57	±	0.47
5.	*cn en*^{1}*/pwn en*^{1}	5.04	±	0.47	12.56	±	0.83
6.	*cn en*^{1}*/Df(2)en*^{21}	5.33	±	0.32	12.97	±	0.70
7.	*cn en*^{1}*/Df(2)en*^{30}	5.80	±	0.50	13.50	±	0.85
8.	*pbx/Df(3)P9*	0.11	±	0.66	12.56	±	5.20
9.	*T(2;3)Hm/SM5*	0.00			17.90	±	5.65

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

[illegible]

Figure 6-9. The genetic map of the proximal Bithorax Complex (Lewis, 1963).

Figure 6-10. Allotypic wing and notum produced in the genotype *bx*^{3}/Df(3)P9. Df(3)P9 is a deficiency for the entire Bithorax Complex kindly supplied by E.B. Lewis. Note the complete duplicate notum and anterior wing including veins I, II, and III, which replace the metanotum and haltere

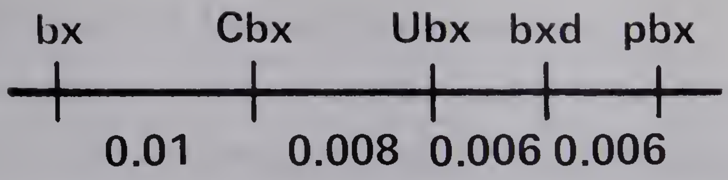


Fig. 6-9

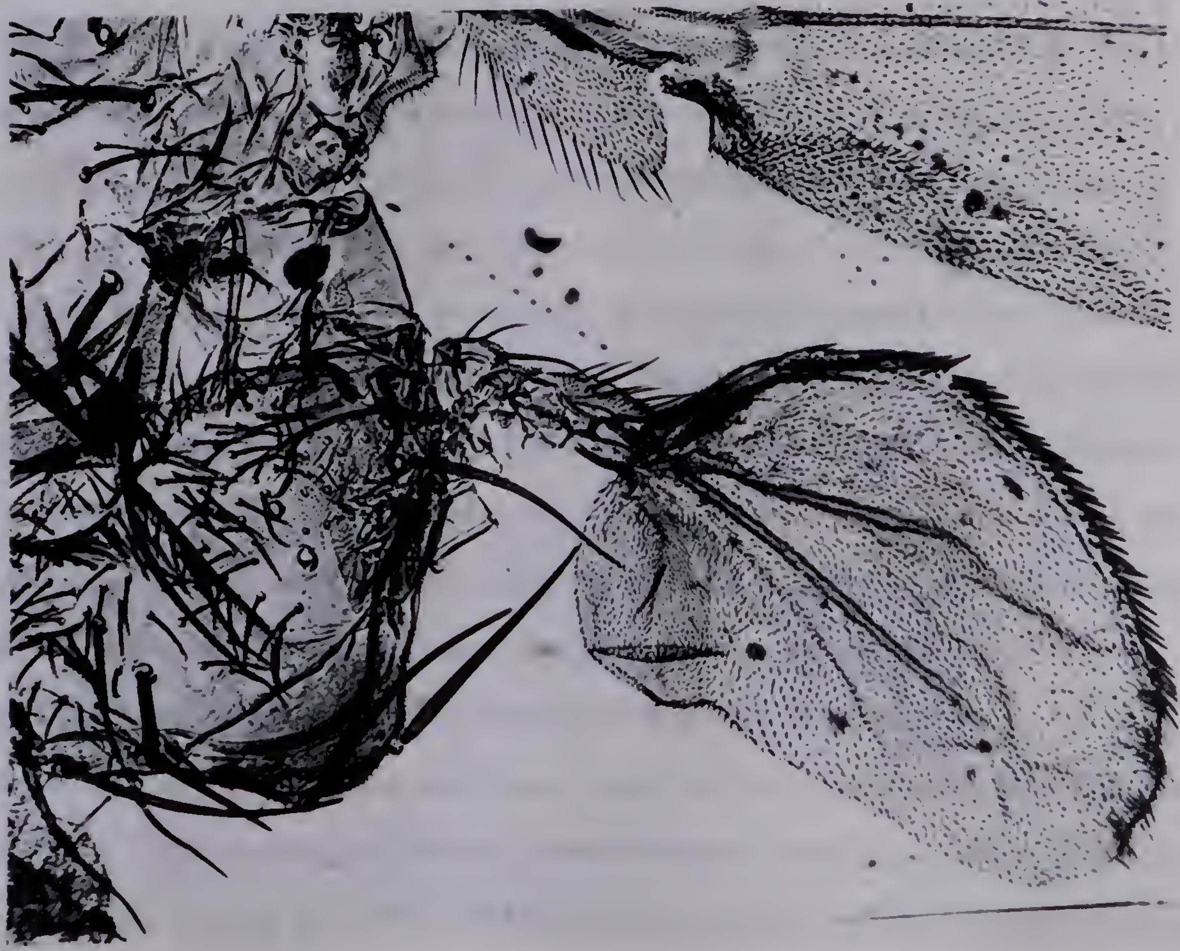


Fig. 6-10

of function will be described first, followed by neomorphs.

bithorax (*bx*). A number of mutants at the *bx* locus have been isolated. All behave as hypomorphs, and have been described as causing the transformation of the anterior compartment of the metathorax to that of the mesothorax (Lewis, 1955; Garcia-Bellido, 1975a). In the dorsal metathorax, notum and anterior wing structures appear in place of metanotum and anterior haltere. The effects of different hypomorphic alleles are most strongly expressed in specific regions but the strongest allele, bx^3, over a deficiency for the locus causes a virtually complete transformation (Morata and Kerridge, 1980). Figure 6-10 shows one such example. A complete notum and anterior wing blade are present, but no posterior-specific mesothoracic structures are seen. In mild *bx* transformations the posterior haltere is unaffected. I have found, however, that in extreme transformations the posterior haltere may exhibit either of two phenotypes. In general, the haltere tissue is continuous with the adjacent wing tissue. In addition, the trichomes on the dorsal surface are noticeably more widely spaced than those on the ventral surface, seeming to be intermediates between wing and haltere trichomes (see Morata, 1975, and below), and the dorsal clump of posterior sensilla is either absent altogether or represented by a single sensillum (Figure 6-11). On the ventral surface the trichomes appear typical of the haltere, and a few sensilla are generally found (Figure 6-12). In rare cases the posterior haltere tissue is not continuous with the wing tissue but forms a separate vesicle. In such cases the dorsal surface invariably carries a large clump of sensilla (Figure 6-13). Both phenotypes have been found with more than one allele (bx^3 and bx^7). Taken together, they seem to indicate an effect of mutants at the *bx* locus in the posterior compartment. Lewis (1955, 1963) has noted a very slight transformation of the posterior region of the haltere to wing in bx^3 +/+ *pbx* heterozygotes under extreme conditions, which he attributes to an effect of *bx* mutation on the activity of the *pbx* locus (see below) of the same chromosome, rather than to an intrinsic effect of the *bx* locus. I shall assume that this is the case (see Molecular evidence, section 7, for discussion), but the alternative has not been demonstrated to be false.

In the third leg even alleles which are weak with respect to haltere phenotypes cause complete transformation of anterior metathoracic markers to those of the anterior mesothorax (Table 3, lines 1-3). This difference may indicate either differing amounts of

Figure 6-11. The dorsal surface of haltere tissue in a *bx*^{3} homozygote. Note the lack of sensilla and the comparatively large size and wide spacing of the trichomes (cf. Figure 6-12).

Figure 6-12. The ventral surface of the haltere tissue in Figure 6-11. Note the three sensilla and more closely spaced trichomes.

Figure 6-13. The dorsal surface of haltere tissue of genotype *bx*^{3}/Df(3)P9. Note the unintegrated haltere tissue and the numerous sensilla (cf. Figure 6-12).

Fig. 6-11

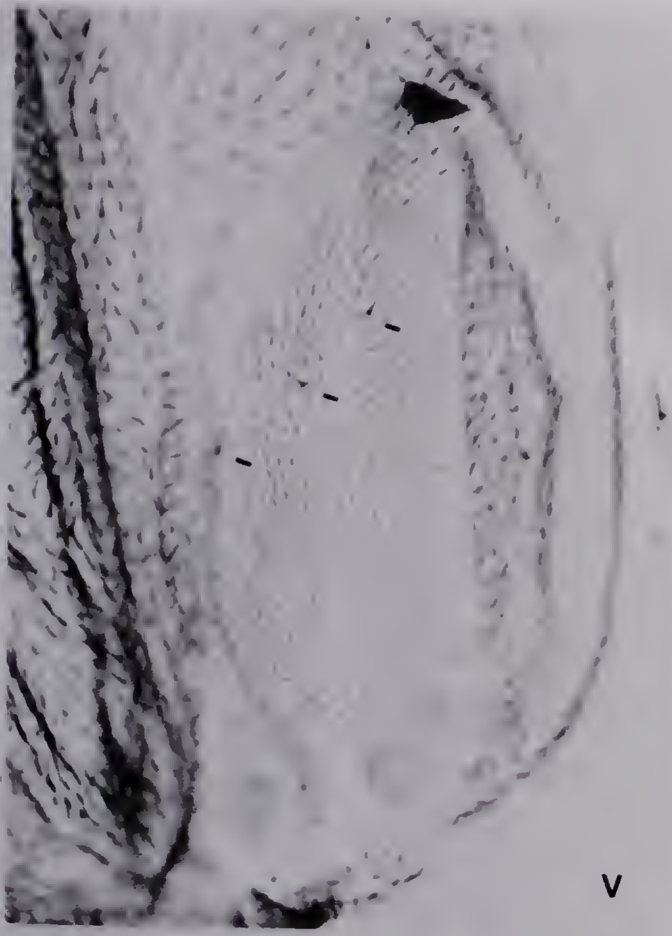


Fig. 6-12

Fig. 6-13

Table 3. Incidence of various leg markers in a series of Bithorax Complex genotypes.

	LEG	GENOTYPE	N	SP	ThB	EB	AB	CoP	BH^-	PrB	R3SB	Tsp	R2	Y	DB	H^-B	R3TB	TR	PR
1.	3	bx^{3}/bx^{3}	22	22	22	22	22	--	1	--	--	--	--	22	2	22	22	22	22
2.	3	$bx^{3}/Df(3)P9$	18	18	18	18	18	--	8	--	--	--	--	18	--	18	18	18	18
3.	3	bx^{34}/bx^{34}	20	20	20	20	20	--	--	--	--	--	--	20	2	20	20	20	20
4.	3	*pbx/pbx*	14	--	--	--	--	14	14	14	14	10	14	--	--	--	--	--	--
5.	3	*pbx/Df(3)P9*	18	--	--	--	--	18	18	18	18	18	18	--	--	--	--	--	--
6.	3	*bxd/Df(3)P9*	14	--	--	--	--	--	10	13	14	--	--	12	2	--	--	13	14
7.	4	*bxd/Df(3)P9*	14	--	--	--	--	--	--	--	8	--	--	--	1	12	1	14	14
8.	3	*pbx/bxd*	16	--	--	--	--	--	14	12	16	--	1	16	7	--	--	16	15
9.	3	$bx^{3}pbx/bxd$	30	--	--	--	--	--	28	14	11	--	--	30	8	18	7	30	30
10.	3	bx^{3}/bxd	18	--	--	--	--	--	--	--	--	--	--	18	1	18	18	18	18
11.	2	*Cbx/Cbx*	15	15	15	15	15	--	15	15	15	15	15	15	--	--	--	--	--
12.	2	*T(2;3)Hm/+*	17	17	17	17	17	17	17	17	17	17	17	--	--	--	--	--	--
13.	3	*T(2;3)Hm/pbx*	28	--	--	1	--	18	28	25	28	14	8	11	2	4	--	9	20
14.	3	*T(2;3)Hm/bxd*	10	--	--	--	--	10	10	10	10	7	10	--	--	--	--	3	--
15.	4	*T(2;3)Hm/bxd*	5	--	--	--	--	--	--	--	--	--	--	--	--	5	5	5	5

residual *bx*$^-$ activity in the two discs or differing minimal requirements of *bx*$^+$ activity to prevent transformation in the two discs. The latter interpretation is consistent with the transformations seen in the *Cbx* mutation and with the results of regeneration studies (Tiong, 1982; see below).

postbithorax (*pbx*). Two alleles are known at the *pbx* locus. The original allele was isolated in the same chromosome as the *Cbx* mutation (see below) from which it was later separated by recombination (Lewis, 1954). In *pbx* mutants the posterior haltere region including dorsal sensilla are replaced by structures of the posterior wing while anterior markers are unaffected (see Figure 6–14, and Table 2, line 8). Similarly, posterior third leg markers are completely replaced by posterior second leg markers (Table 3, lines 4–5). The *pbx* transformation of the posterior compartment is thus analogous to that of *bx* in the anterior compartment. In addition, *pbx* third legs have a thin but recognizable strip of tissue devoid of trichomes running along the anterior–posterior boundary between rows 3 – 4 in the femur (S. Tiong, personal communication); the bald strip is seen in neither second nor third legs, and its significance in mosaic legs is unclear.

While both *bx* and *pbx* cause transformations of metathorax to mesothorax, their domains and ranges appear to be mutually exclusive and to correspond to the anterior and posterior compartments of the segments, respectively. In the wing hinge the A–P boundary is undefined, but there is no overlap in the sets of elements produced in the range by the *bx* and *pbx* mutations (Adler, 1978a), which is consistent with their being compartment specific. With respect to the other criteria for potential control genes, they show no maternal effects, they behave autonomously (Garcia–Bellido and Lewis, 1976; Morata and Garcia–Bellido, 1976) and the wild–type activity is required in normal development from some time before the first larval instar to until the late third instar (Morata and Garcia–Bellido, 1976). The *bx* locus thus appears to be the control gene for the anterior metathoracic compartment and the *pbx* locus that for the posterior metathoracic compartment. They cause the "one to one" transformations of pattern units expected of hypomorphs in an epistatic ideographic system. The existence of separate control loci for the anterior and posterior compartments of the metathorax is also consistent with the earlier conclusion that the *en* gene is not involved in the determination of the metathoracic compartments. The anterior and posterior compartments of the

Figure 6-14. Allotypic wing structures produced in the genotype *pbx*/Df(3)P9. Note the alar lobe (AL) and posterior row (PR). Note also the sensilla in the anterior compartment.

Figure 6-15. A supernumerary haltere formed in place of a first abdominal segment in a *bxd* homozygote. Note the partial transformation of posterior metathorax to posterior mesothorax in the autotypic haltere.

Figure 6-16. Four legs produced by the genotype *bxd*/Df(3)P9. Note that the transverse rows (TR) in the fourth leg are more developed than those in the third, indicating a less severe *pbx*-like transformation in the fourth leg (see Table 3, lines 6 and 7).

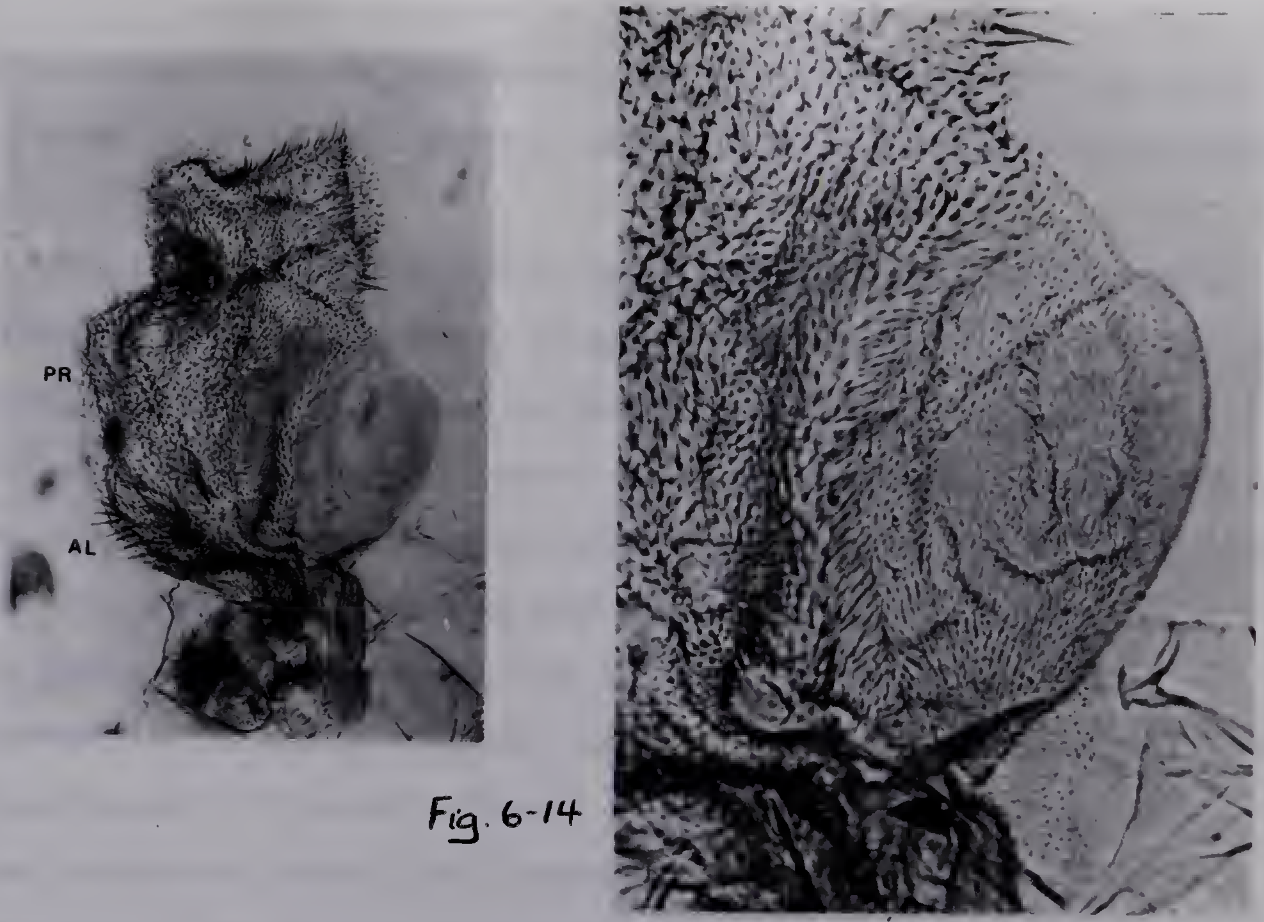


Fig. 6-14

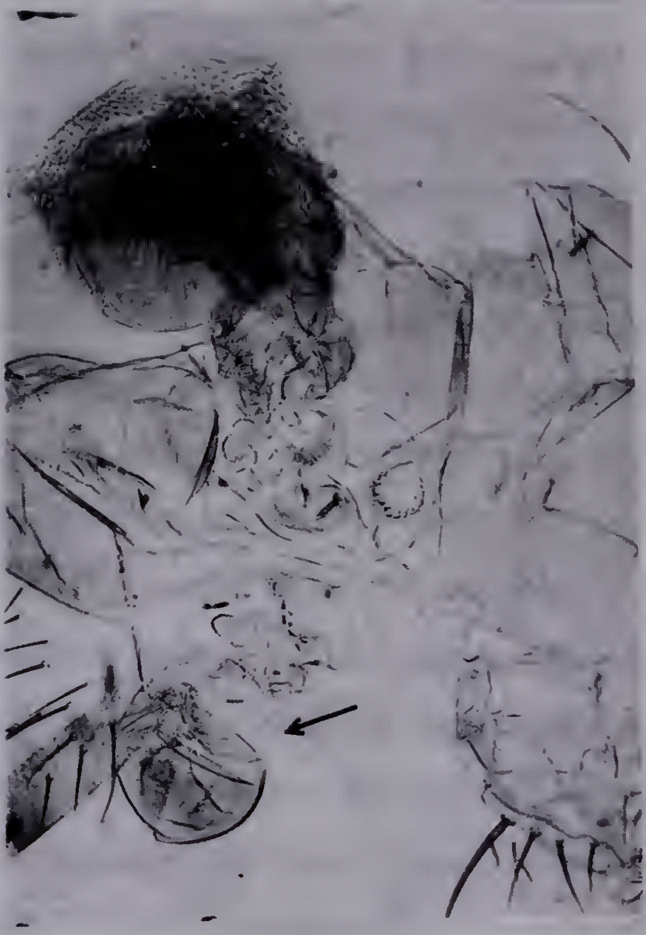


Fig. 6-15

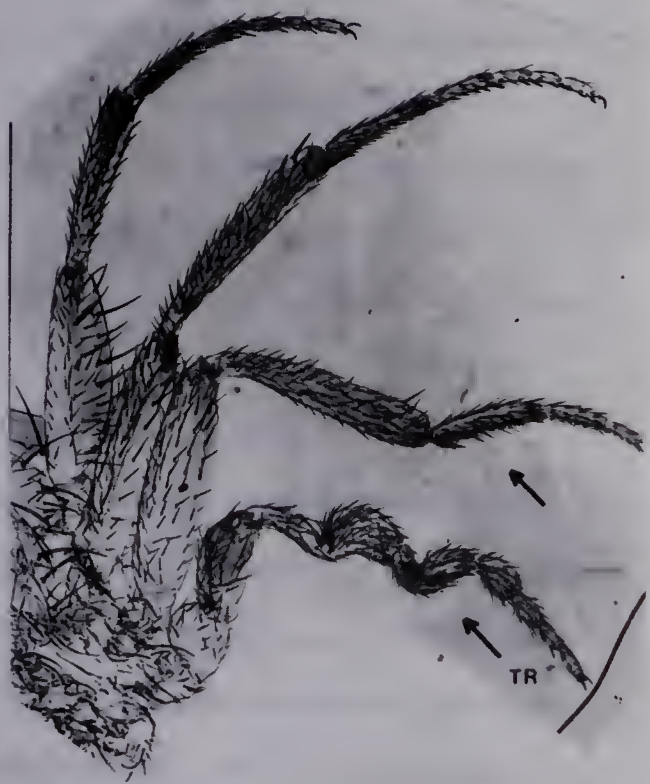


Fig. 6-16

metathorax appear each to be determined by the ON state of a different unique gene rather than by the ON state of a common "metathoracic" gene and the differing ON/OFF states of the *engrailed* gene.

bithoraxoid (*bxd*). Mutations at the *bithoraxoid* locus are either hypomorphic point mutants or presumably amorphic breakpoints. Their effects are best described as a transformation of the first abdominal segment to metathorax, and an additional transformation of the posterior metathorax to posterior mesothorax. The abdominal transformation is mainly restricted to the absence of the first tergite and the appearance of a fourth leg or pair of legs ventrally. Only very rarely is a supernumerary haltere formed (less than 0.01% of cases, Kerridge and Sang, 1981; see Figure 6–15). The transformation of the autotypic posterior metathorax to posterior mesothorax is always weak (see Figures 6–15 and 6–16, Table 3, line 6), and the allotypic legs are generally even less transformed (Table 3, line 7) (Kerridge and Sang, 1981). The double heterozygote *bxd +/+ pbx* shows only the *pbx*–like transformation, while *bx +/+ bxd* is wild–type (Table 3, lines 8–10). It has been shown that the effects of *bxd* in the abdomen are autonomous and that the wild–type product is required until pupariation (Morata and Garcia–Bellido, 1976). The transformation of posterior metathorax to posterior mesothorax has again been ascribed to an effect on the neighboring *pbx* locus rather than to an intrinsic effect of the *bxd* locus (Lewis, 1955). Accepting this conjecture for the moment (see Molecular evidence, section 7), the *bxd* locus may be said to cause a "one to one" transformation of the first abdominal segment to metathorax, and thus be the control gene for the first abdominal segment in an epistatic ideographic system.

Ultrabithorax (*Ubx*). *Ubx* mutations in heterozygous condition cause the capitellum of the haltere to be slightly enlarged and to carry a few bristles in the anterior region. This dominant effect is due to a haplo–insufficiency of the locus, as deficiencies of the locus produce the same effect. *Ubx* mutations are homozygous lethal but are cell–viable in somatic clones. In such clones they transform the metathorax and first abdominal segment to mesothorax (Morata and Garcia–Bellido, 1976; Morata and Kerridge, 1981, but see Chapter IX). While this "many to one" transformation suggests that the *Ubx*$^+$ locus may be a control gene in a cumulative ideographic system (see Figure

5–5), the interactions of *Ubx* mutants with the other Bithorax Complex mutations discussed thus far argue otherwise. As Lewis elegantly showed (Lewis, 1955), the *trans*–double heterozygotes between *Ubx* and *bx*, *pbx*, or *bxd* show the extreme phenotype of the recessive allele as well as the *Ubx* heterozygous effect, while the *cis*–double heterozygotes show the *Ubx* effect alone. Thus, *Ubx* mutations are equivalent to *cis*–inactivations of the *bx*, *pbx*, and *bxd* loci, but have no effect on loci located on the homologous chromosome. This *cis*–specificity would not be expected of a gene coding for a diffusable product which interacts with downstream loci. It has been suggested that this behavior is more compatible with *Ubx*$^+$ being an attribute of the chromosomal region necessary for functioning of the *bx*$^+$, *pbx*$^+$, and *bxd*$^+$ genes, rather than a control gene of the type we have been discussing. (Garcia–Bellido, 1975; Hayes *et al.*, 1979).

Two neomorphs exist which transform mesothoracic structures to metathoracic ones. They will be described, and their effects interpreted in terms of the activation of the *bx*$^+$ and *pbx*$^+$ loci.

Contrabithorax (*Cbx*). *Contrabithorax* is a dominant neomorph which arose simultaneously with the *pbx*^{1} mutation (Lewis, 1954). In extreme cases it may cause an almost complete transformation of wing to haltere and greatly reduce the amount of notum. In general, the most posterior region of the wing is always transformed and the probability of transformation of a given wing structure decreases in an anterior direction (Morata, 1975). It has been assumed that the transformation results from the activation of the *bx* and *pbx* loci in the mesothorax (Lewis, 1963). The dorsal and ventral sensilla groups are present whenever their sites are included in the region transformed (see Figures 6–17 and 6–18). There are several interesting aspects to this transformation. First, by combining *Cbx* with various other mutations in the Bithorax Complex Lewis (1964) and Morata (1975) have shown that although *Cbx* causes an activation of *bx* and *pbx*, in the mesothorax, it also causes a hypo–function of *bx* and *pbx* in both meso– and metathoraces. In *Haltere mimic* (*Hm*) (see below) and in various mutations in the Antennapedia Complex (Denell *et al.*, 1981) an association between abnormal activation and hypo–function is also seen. Second, it was found that clones induced could include both wing and haltere tissue until very late in development (Morata, 1975). This suggests

[illegible]

Figure 6-17. The dorsal surface of a wing transformed by *Cbx*. Note the clump of sensilla in its appropriate location.

Figure 6-18. The ventral surface as in Figure 6-17. Note the beginning of the line of sensilla in its appropriate location.

Figure 6-19. Triple row bristles completely surrounded by haltere trichomes in an extreme *Cbx* transformation (cf. Figure 8-10).

Fig. 6-17

Fig. 6-18

Fig. 6-19

either that the activation of *bx* and *pbx* in the mesothorax is a very late event, or that they are activated in the mesothorax early and there is a certain probability of loss of activity during development, perhaps due to the hypofunction of the alleles (Morata, 1975). The fact that the wing and notum may be transformed completely into haltere and metanotum in extreme cases indicates that cell number is regulated as well as cell phenotype, and strongly argues against a late decision to activate *bx* and *pbx*. Third, the characteristics of the interface between wing– and haltere–determined cells may vary in interesting ways. Cells with trichomes intermediate in phenotype between those of wing and of haltere may be found (as noted above in *bx* mutations), and in cases of extreme transformations, wing bristles of the anterior margin may be completely surrounded by haltere trichomes (Morata, 1975) (see Figure 6–19). The significance of this last phenotype will be apparent in section 8.

The mesothoracic leg remains almost completely untransformed, which is consistent with the interpretation made earlier that leg discs require a higher level of bx^+ activity than dorsal discs to be metathoracic (Table 3, line 11)

Haltere mimic (*Hm*). *Haltere mimic* is associated with the complex translocation T(2:3)*Hm* which contains a break point at 89 E2–3, the region of the Bithorax Complex (Lewis, personal communication). It has no effect on the notum or proximal wing hinge, but transforms the distal wing into haltere in an almost certainly compartment specific way. The anterior compartment of the distal wing is completely transformed to haltere. It contains the more distal part of the curving line typical of the anterior compartment (see Table 2, line 9). The posterior compartment is only partially transformed, having trichomes of intermediate size and spacing characteristics and no sensilla (see Figure 6–20). This suggests that *Hm* causes both *bx* and *pbx* to be activated in the mesothorax, but also causes a hypo–function of *pbx*. This interpretation was tested by constructing flies of the genotype bx^3 *pbx*/T(2:3)*Hm*. The bx^3 and *pbx* mutations are completely recessive, which allows one to ascribe any transformation of haltere to wing to hypo–function of the loci on the *Hm* chromosome. As can be seen in Figure 6–21 in bx^3 *pbx*/T(2:3)*Hm* flies the distal meso– and metathoracic appendages have virtually indistinguishable phenotypes: the anterior compartment is entirely haltere–like while the posterior compartment has mixed wing–haltere characteristics, supporting the original

Figure 6–20. Wing and haltere derivatives in T(2:3)*Hm*/+. Note the difference in size and spacing characteristics of the trichomes in the anterior and posterior compartments.

Figure 6–21. Wing and haltere disc derivatives in the genotype *bx*^{3} *pbx*/T(2:3)*Hm*. Note the alar lobes and hybrid wing–haltere characteristics of the posterior wing blade and capitellum. Note also the similar line of sensilla.

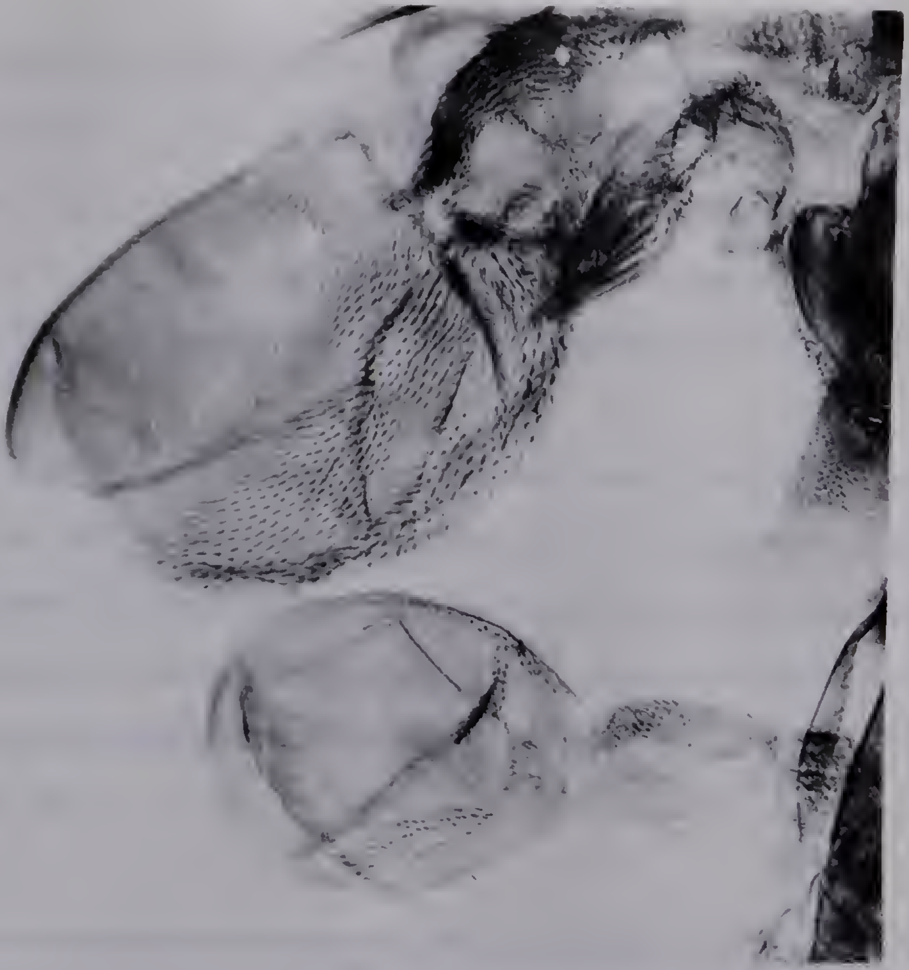


Fig. 6-20

Fig. 6-21

interpretation. That the consistent difference between anterior and posterior may be attributable to differential activity of the *bx* and *pbx* loci becomes important in section 8.

The second legs in *Hm*/+ flies are completely untransformed (Table 3, line 12). The third legs of *Hm*/+ are also completely untransformed, but in *Hm*/*bx*^{3} *pbx* flies the posterior compartment is partially transformed to mesothorax as in the haltere (Table 3, line 13).

Flies of the genotype *Hm*/*bxd* show a partial transformation of posterior third leg to posterior second leg as would be expected from the reduction of *pbx*$^+$ activity by the *bxd* allele. In addition, the first abdominal segment is gone, and a fourth leg may appear, indicating that *Hm* also causes a lessening or loss of *bxd* function. The fourth legs are always less transformed to mesothorax than the third legs, as in *bxd* fourth legs (Table 3, lines 14–15).

Summary

By their domains, the amorphic and hypomorphic mutations discussed above appear to identify control loci of a mixed combinatorial–ideographic system. The *engrailed* gene may possibly be a gene which originally controlled determination in a combinatorial way in all segments and which still functions in the pro– and mesothoraces, but whose role in determination has been superceded in the metathorax and first abdominal segment. The *bithorax* and *postbithorax* genes act as epistatic control genes for the anterior and posterior compartments of the metathorax, and the *bithoraxoid* gene acts as an epistatic control gene for the first abdominal segment. In the next chapter, the information provided by the ranges of these mutations will be discussed in detail, leading to the elucidation of the transcriptional control of each gene and, as a consequence, to a proposal for the genetic fine structure of the proximal Bithorax Complex. The available molecular evidence will then be reviewed, as well as some other models for the functioning of the Bithorax Complex in development.

VII. A Model of Genetic Interaction

The transformations caused by amorphic and hypomorphic mutations discussed above are all anteriorly directed and eventually culminate in the anterior mesothorax (see Figure 7–1). Gross deficiencies for the Bithorax Complex are lethal, but survive to the late embryonic or early first instar stage. Such larvae also exhibit anteriad transformations, and larvae deficient for the entire Bithorax Complex have the metathorax and all abdominal segments transformed to the mesothorax (Lewis, 1978). The mesothorax thus represents the "primitive level" (Lewis, 1955, 1978) or "developmental sink" (Garcia–Bellido, 1975) where all genes of the Bithorax Complex are OFF. With this in mind, the ranges of the *en*, *bx*, *pbx*, and *bxd* mutants may be each interpreted in terms of ON/OFF states at the other loci in the compartments of the meso– and metathorax and the first abdominal segment.

A. The Control of Transcription

The following account may be best understood by reference to Figure 7–2. In the mesothorax all the genes of the Bithorax Complex are OFF. The *engrailed* transformation of posterior to anterior mesothorax has been interpreted as evidence that *en* is normally ON in the posterior and OFF in the anterior mesothorax (Lawrence and Morata, 1976). In the metathorax the activity states of the *en*, *bx*, and *pbx* loci may be inferred from the ranges of bx^- and pbx^- mutants. *Bithorax* mutants transform the anterior mesothorax to the developmental sink, suggesting that all other loci are OFF in the anterior metathorax. *Postbithorax* transforms the posterior metathorax to posterior mesothorax, suggesting that in the absence of pbx^+ product the *en* gene is ON and all other Bithorax Complex genes are OFF in the posterior metathorax. Finally, *bxd* mutants transform the first abdominal segment to metathorax, suggesting that the *en*, *bx*, and *pbx* loci are all ON in their respective compartments in the first abdominal segment. Two observations (Lewis, 1978) suggest that the activity pattern of the first abdominal segment is reiterated in all abdominal segments. First, in larvae deficient for the entire Bithorax Complex except the region from *bx* to *Ubx*, all larval abdominal segments exhibit characteristics of the

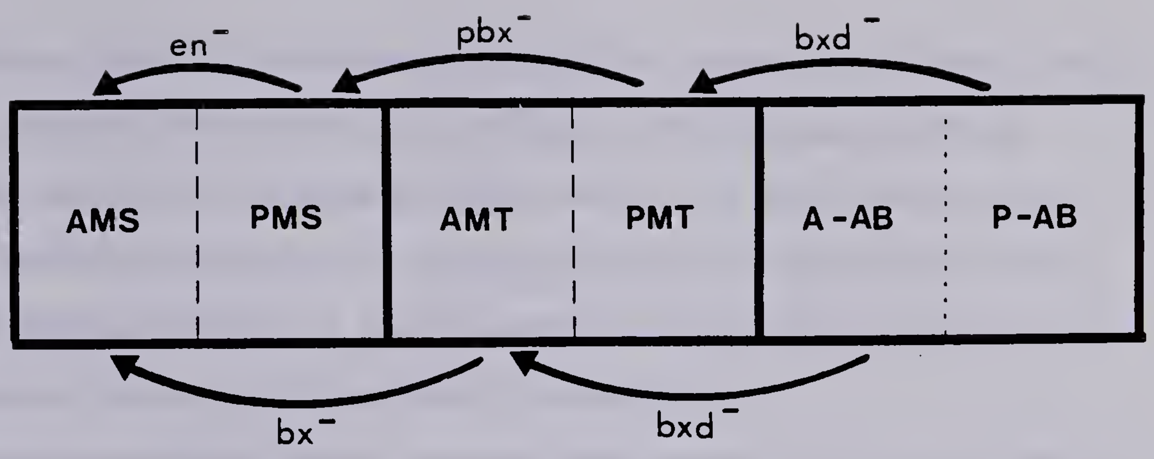


Figure 7–1. The transformations caused by *en* and amorphic and hypomorphic mutants of the proximal Bithorax Complex.

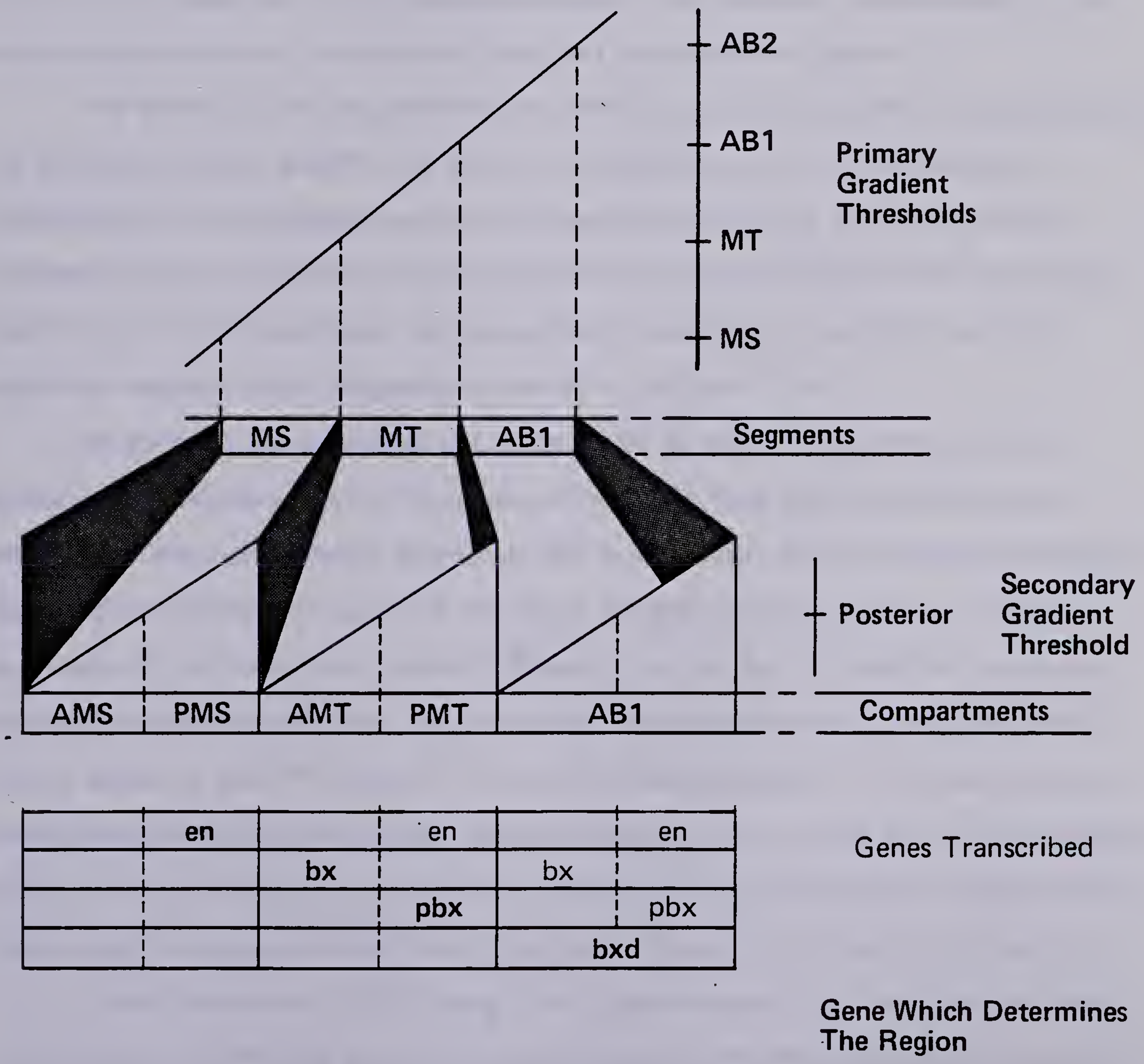


Figure 7–2. The genes which are thought to be ON in the various pattern units.

anterior metathorax rather than the anterior mesothorax, indicating the activity of the *bx* locus in these segments. Second, although *bxd* has no effect on adult abdominal segments other than the first, in larvae a thoracic marker -- a pair of ventral pits -- appears on all abdominal segments. This indicates that *bxd* is normally active in these segments to suppress this marker, a situation which may be formally equivalent to the continued effects of *en* on enzyme markers in the metathorax.

The tabulation of active control genes by compartment reveals spatial patterns in the way these genes are themselves activated. It appears that two levels of positional information are necessary to account for the patterns, a primary level determining segmental position within the embryo and a secondary level relating to anterio-posterior position within segments which establishes anterior and posterior compartments. The control relationships of the individual genes are diagrammed in Figure 7-3.

The activity of the *en* gene may be seen to be governed strictly by position within the secondary field. It is OFF in the anterior compartment and ON in the posterior compartment of all segments we have considered (Figure 7-3a). In a similar though different fashion, the activity of *bxd* is governed strictly by position within the primary field. It is OFF in the metathorax and all segments anterior to it, and ON in the first abdominal segment and all segments posterior to it (Figure 7-3b).

By contrast, the control of activation of the *bx* and *pbx* genes involves both primary and secondary levels of positional information. Both genes are OFF in the mesothorax and more anterior segments. Each is potentially ON in the metathorax and all segments more posterior, but *bx* is ON only in the anterior compartments of these segments and *pbx* only in the posterior (Figure 7-3c). As the ON states of *bx* and *pbx* summarize locations within two separate fields, they are equivalent to the ideographic control genes "g" and "h" in Figure 4-3. That they act epistatically -- i.e., that there is no developmentally stable "metathorax" gene equivalent to "e" in Figure 4-3 -- was deduced above, and is further shown by two experiments involving regeneration in imaginal discs. These experiments also indicate novel features of the control of the *bx* and *pbx* loci.

Tiong (Tiong *et al.*, 1977; Tiong, 1982) used a temperature-sensitive cell lethal system (Russell, 1974; see above) to cause cell death in the third leg discs of second instar larvae; this produces disc fragments which duplicate *in situ*, yielding adult flies

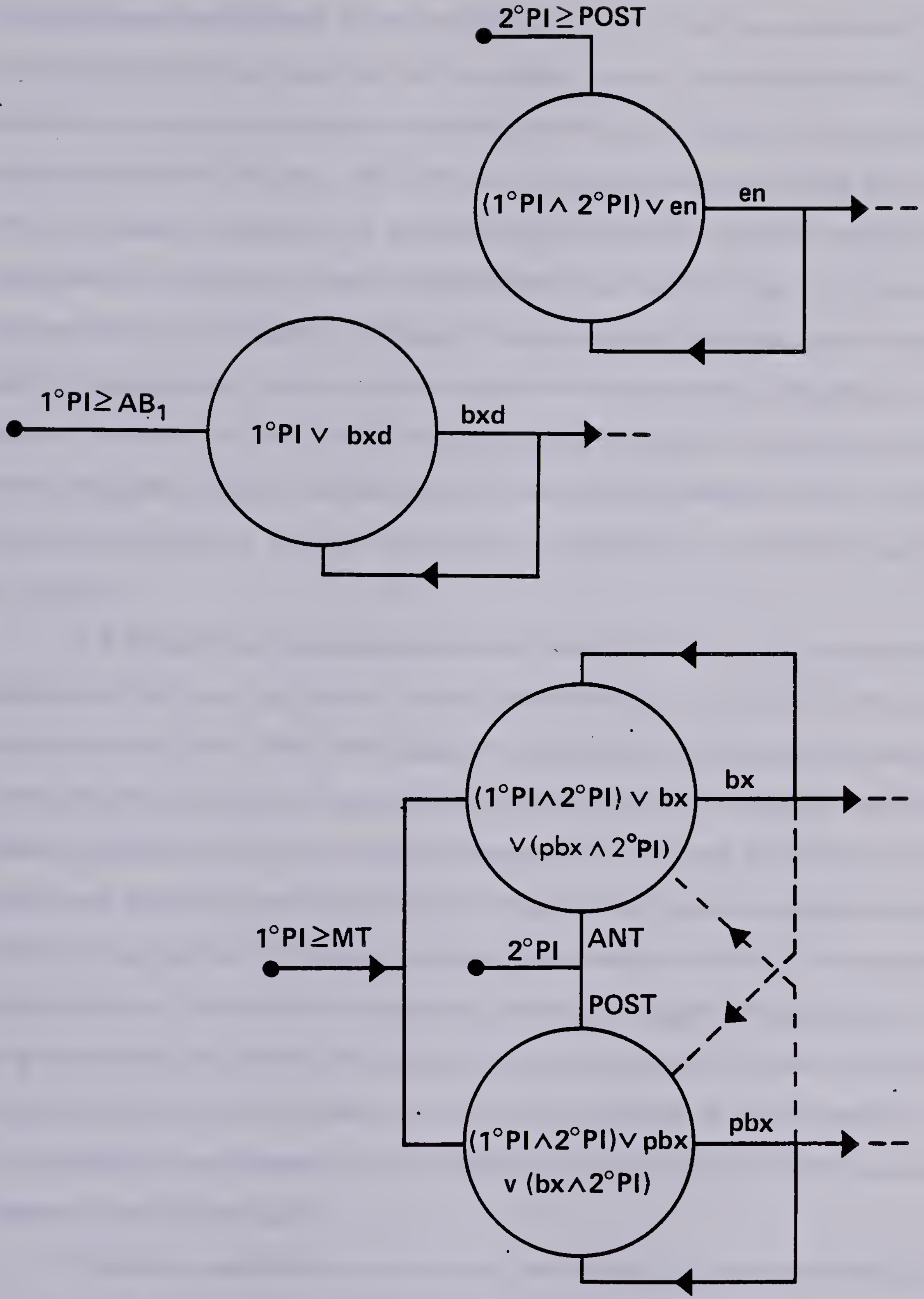


Figure 7–3. Gene control networks (adapted from Wolpert and Lewis, 1975. Circles represent genes and arrowed lines represent control connections. The name of each gene is written to the right of the circle while the conditions allowing transcription of the gene are written within the circle. "g h" is read as "either g or h is present, or both are present"; "g h" is read as "both g and h are present. (a) To be transcribed initially, the *en* gene requires that the secondary gradient level exceed the posterior threshold (2 PI POST); it subsequently promotes its own transcription: (2 PI *en*) (b)The *bxd* gene is transcribed at blastoderm in response to primary gradient levels exceeding the threshold of the first abdominal segment, and thereafter promotes its own transcription. (c) Primary gradient levels exceeding the metathoracic threshold level (1 PI MT) allow either *bx* or *pbx* to be transcribed depending on the level of the secondary gradient; each product then feeds back to maintain its own transcription. See text for discussion.

with duplicated legs (Russell, Girton and Morgan, 1977). It is known that cells from the prospective anterior compartment of the original leg may produce both anterior and posterior compartment structures in the duplicate upon inclusion in the regeneration blastema (Girton and Russell, 1981). Flies of the genotype $bx^{-}pbx^{+}$ have third legs in which the anterior metathorax is completely transformed to anterior mesothorax while the posterior metathorax remain untransformed (e.g. Table 3, lines 1–3). When discs of this genotype were caused to duplicate it was observed that in the duplicate both anterior and posterior compartments were often transformed to mesothorax, implying a failure to activate the pbx^{+} locus when the duplicate posterior compartment was formed. As the duplicate posterior compartment is never transformed in $bx^{+}pbx^{+}$ flies, the results imply that activation of the pbx^{+} locus during regeneration requires the presence of the bx^{+} allele.

In a series of analogous experiments, Adler (1978b) tested the regenerative capacities of $bx^{+}pbx^{-}$ and $bx^{-}pbx^{+}$ haltere discs during *in vivo* culture. When posterior fragments of $bx^{+}pbx^{-}$ discs were caused to regenerate it was found that anterior tissue derived from the posterior fragment was also transformed to mesothorax, implying a failure to activate the bx^{+} locus during regeneration. As similar fragments from $bx^{+}pbx^{+}$ discs never generate transformed anterior tissue under similar conditions, the results indicate a requirement for the pbx^{+} allele in the activation of the bx^{+} locus during regeneration. In the companion experiment, testing the regenerative capacity of anterior fragments of $bx^{-}pbx^{+}$ discs, the results were the opposite of those of Tiong *et al.*, i.e., posterior structures were never transformed. The difference may be merely a reflection of the differing requirements for bx^{+} product in the third leg and haltere developmental systems as noted previously.

These two experiments indicate that the memory of the metathoracic commitment in the primary field resides in the ON states of the bx^{+} and pbx^{+} loci; during regeneration the primary gradient is no longer extant, and the ON state of one locus is therefore required for the activation of the other when a change in compartment status occurs. These relationships are shown by the dashed lines in Figure 7–3c.

B. The Fine Structure of the Complex

A Fine Structure Model

The proposed conditions under which the *bx* and *pbx* loci are activated contain a potential contradiction: the two loci are said to be activated in a mutually exclusive fashion which might imply some form of negative interaction between the product of one gene and the control region of the other, yet during regeneration a positive interaction between these same compartments is found. Were the *bx* and *pbx* loci to map far apart a very complex scheme of interactions would be required to resolve this dilemma. However, the two loci map very close to each other, and we have suggested that the interactions between them may involve structural as well as kinetic elements (Hayes *et al.*, 1979).

Figure 6–9 shows the genetic map of the proximal region of the Bithorax Complex. *Cbx* and *Ubx* have been interpreted above as mutations affecting the control of transcription of both the *bx* and *pbx* loci; that the control mutants map between the structural loci suggests that transcription of the two loci proceeds in opposite directions from a common central control region (see figure 7–4). The wild–type activation of the *bx* and *pbx* loci is envisaged to be a two–step process. In segments anterior to the metathorax the chromosomal region containing the common control site for *bx* and *pbx* would remain "closed", or inaccessible to transcription. In the metathorax and all segments more posterior the control region would become "open", but only conditionally so, in response to a primary positional information gradient. Upon the establishment of secondary positional information gradients within segments, transcription would be initiated at open promoters, toward the *bx* locus when secondary gradient levels were below a critical threshold value and toward the *pbx* locus when levels exceeded that value. If transcription in one direction precludes transcription in the other, the activation of *bx* and *pbx* loci would be mutually exclusive events. Control regions similar to that proposed are known in a number of systems (Ptashne *et al.*, 1976; Zieg *et al.*, 1977; Hirsh and Schleif, 1977).

Surgical experiments on mature discs have indicated that even late in development cells may still read their global position within the disc (Bryant, 1975). This means that the gene products of the *bx* and *pbx* loci need only feedback to maintain the common

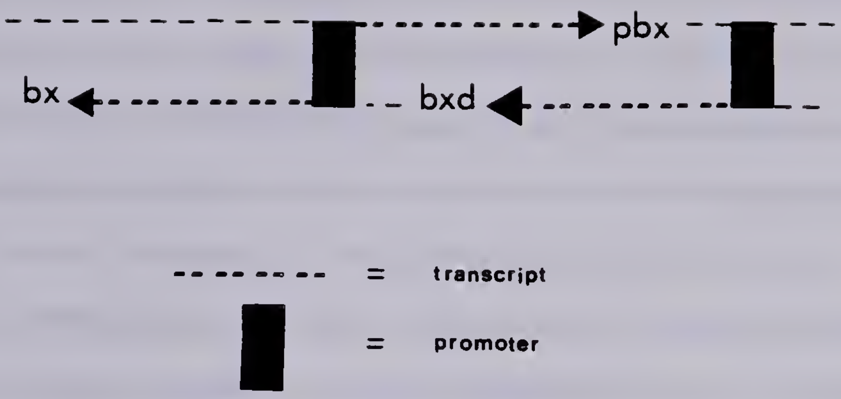


Figure 7–4. The proposed control regions and directions of transcription of the *bx, pbx,* and *bxd* loci.

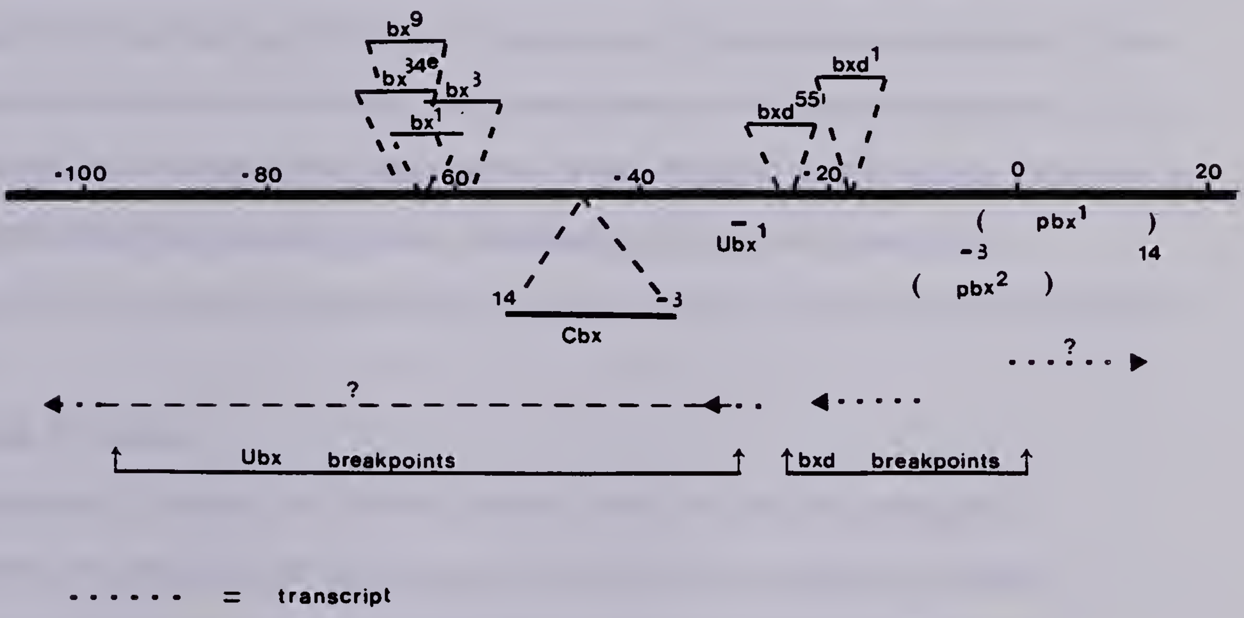


Figure 7–5. The molecular evidence (W. Bender, M. Akam, personal communication).

control region in a stably "open" state, and that the direction of continued transcription will be determined by the persistent secondary gradient. Thus, during development the chromosome structure corresponding to the "open" state of the common promoter region would constitute a memory of the metathoracic decision taken at blastoderm.

The results of both Tiong and Adler may be understood in terms of the model as being due to secondary "closure" of the control region. In wild–type metathoracic discs prospective anterior compartment cells would have open promoter regions due to feedback from the *bx*$^+$ product; a change in compartmental specificity during regeneration would be accompanied by a change in direction of transcription toward the *pbx* locus, and the metathoracic commitment would be maintained. In *bx*$^-$ *pbx*$^+$ discs however the control region in anterior cells may have become "closed" due to insufficient feedback from the mutant product. The *pbx*$^+$ locus would thus be inaccessible when such cells enter a regeneration blastema, and posterior structures would also be transformed to mesothorax.

The *bxd* locus maps between the proposed control region and the *pbx* locus. As it is clearly under separate developmental control from *pbx* (Figure 7–3), it most probably is transcribed from a control region to the right of *pbx* (see Figure 7–4). The interweaving of the *bxd* and *pbx* loci in this fashion is offered as an explanation of the effects of *bxd* mutants on *pbx* activity, as breakpoints at *bxd* would remove *pbx* from its control region. It is necessary to assume however, that *bxd* point mutants interfere in some way with either the transcription or processing of the *pbx* product. An arrangement of this kind could have evolved by a process of gene duplication (Hayes *et al.*, 1979).

The Molecular Evidence

The Bithorax Complex has recently been cloned (W. Bender, personal communication). The data are still preliminary, but they afford insight into certain characteristics of the Bithorax Complex and allow a direct test of some of the predictions of the present model. The data are summarized in Figure 7–5. There are several interesting aspects of the data thus far. The first is the finding that spontaneous "point mutations" in the *bx* and *bxd* loci are caused by the insertion of a particular 7.3 kilobase sequence which has been named "gypsy". It is unclear as yet whether gypsy

sequences are transcribed; it appears, however, that the mutational effects are not simply the result of the insertion of foreign DNA *per se*, because the *suppressor of Hairy wing* mutation suppresses the mutant phenotype of at least one "gypsy" insertion without removing the insertion itself (W. Bender, personal communication). It is easy to envision ways in which "active" insertions might affect chromosome structure in neighboring regions; the attribution of posterior metathoracic transformations by *bx* and *bxd* mutants to effects on the *pbx* locus (see above) is thus greatly strengthened by these findings.

A second finding of interest is that *Ubx* mutations span a region of 70 kilobases, from the site of the original *Ubx* allele to well to the left of the *bx* locus (W. Bender, personal communication). While this result suggests that Ubx^+ function is an attribute of the chromosomal region as suggested above, it has recently been reported that three transcripts ranging in size from 3.7 to 4.7 kilobases hybridize to DNA located at both ends of the *Ubx* region, transcribed from right to left (after Hogness, in Marx, Science, 1981). This suggests the existence of a Ubx^+ product (see Other Models, below) which must nonetheless be *cis*-acting in some fashion in view of the arguments presented above. As yet, no transcripts of the intervening region -- including the *bx* locus -- have been found. Whether this region is spliced out of a 70 kilobase transcript or is deleted from the DNA is not yet known. These preliminary data are inconclusive, and are surprizing from almost any perspective; it is to be hoped that further results will aid in their interpretation.

Data on the location and direction of other transcripts within the complex are also preliminary. It is known that the *bxd* region is transcribed from right to left as predicted by the model. No transcript has been positively identified with the *pbx* region though one with characteristics of a transcript from left to right in this region has been found (M. Akam, personal communication). Indirect evidence that the *pbx* region is transcribed from left to right comes from the nature of the *Cbx* mutant. As noted above, *Cbx* arose simultaneously with *pbx* and was later separated from it by recombination. The amorph *pbx* is a deletion of 17 kilobases of DNA, and the neomorph *Cbx* is an insertion of that same DNA in reverse orientation to the left of the Ubx^1 site (W. Bender, personal communication, see Figure 7-5). These findings are consistent with the *pbx* gene being

normally transcribed from left to right, and with *pbx* DNA being transcribed from right to left from the *Ubx*[1] region, in the *Cbx* mutant. It is not yet clear how other attributes of the *Cbx* phenotype correlate with the molecular data.

C. Other Models of the Bithorax Complex

As the loci of the Bithorax Complex are clearly important in determination and have been studied for up to thirty years, it is perhaps not surprising that many models concerned with their actions in development have been proposed. Here I wish to discuss three models, pointing out specific differences with the model proposed above.

The present state of knowledge concerning the Bithorax Complex is due mainly to the studies of E.B. Lewis over the last thirty years. Lewis (1978) feels that the *Ubx* gene codes for a product which effects a change from the primitive, mesothoracic level of development to the level of the metathorax. The *bx* and *pbx* loci are thought to code for products also involved in effecting this change. The abdominal segments are each thought to be similarly controlled by a single gene located in the distal part of the Bithorax Complex. These genes are regulated by the interaction of their control regions with a single anterio–posterior gradient of repressor within the embryo. The different Bithorax Complex genes are thought to have increasing gradient activation thresholds with proximo–distal position within the complex, such that an additional control gene is activated in each segment from the metathorax to the eighth abdominal segment.

While the correlation of single control genes with particular segments and of the proximo–distal arrangement of genes in the complex with their segments in the fly appears to hold true in the abdomen, both correlations break down in the metathorax unless the *bx* and *pbx* loci are ignored and *Ubx* is equated with metathoracic development. The metathorax is thus a special case, and the model presented in this thesis may be viewed as a specific refinement to the more general model due to Lewis. A major point of disagreement, however, lies in the nature of the *Ubx* gene. While the molecular evidence indicates a product for the *Ubx* gene in accordance with Lewis' ideas, I feel that its *cis*–specific effects are more consistent with it defining a structural characteristic of the chromosomal region. Clearly, more data are required.

Sander (1975b) has proposed a general model for pattern specification in insects which involves two levels of positional information. He proposes that in the first step in pattern formation in insect embryos, a primary longitudinal gradient determines the segmental character. In the second step, new secondary gradients are established within the domains defined by physiological barriers which have appeared between segments; these secondary gradients specify the longitudinal character within segments (Figure 7–6(a–1) and 7–6(a–2). Sander feels that some of the *bithorax* mutants may be explicable in terms of these two levels of gradient information. Specifically, he proposes that they interfere with the proper determinative interpretation of the primary gradient, yet leave the establishment of the segmental boundaries and secondary gradients unchanged. The thresholds which control determined states would thus no longer coincide with segmental boundaries. *bx*⁻ cells in the presumptive anterior metathorax would incorrectly interpret the primary gradient values here as mesothorax while correctly interpreting the secondary gradient as anterior, and thus would differentiate as anterior mesothorax (Figure 7–6, (b–1) and 7–6(b–2). The *Cbx* mutant would be a mutant which causes cells in the mesothorax to interpret the primary gradient incorrectly as metathorax while reading the secondary gradient correctly (Figure 7–6(c–1) and 7–6(c–2). *bxd*⁻ mutants would similarly cause cells in the first abdominal segment to misinterpret the primary gradient as metathorax (Figure 7–6(d–1) and 7–6(d–2).

Sander's model is a plausible explanation of the *Cbx* phenotype, but it does not explain the compartment and segment specificity of the *bx* and *bxd* mutants, nor can it explain the phenotype of the *pbx* mutant. The model predicts that all transformations should be to contiguous segments or compartments, as it is difficult to see how a genetic defect could cause cells to interpret noncontiguous gradient values as equivalent while reading the intervening values as distinct (see Figure 7–7). Both these objections are overcome by assuming instead that *bx*, *pbx*, and *bxd* are control genes directing development within specific pattern units.

Garcia–Bellido has proposed that *bx*, *pbx*, and *bxd* are selector genes for the compartments of the metathorax and the first abdominal segment. However, he also considers *en* to be a selector gene controlling posterior development in all thoracic discs (1975). There is a contradiction inherent in these views: if the ON/OFF state of *en*

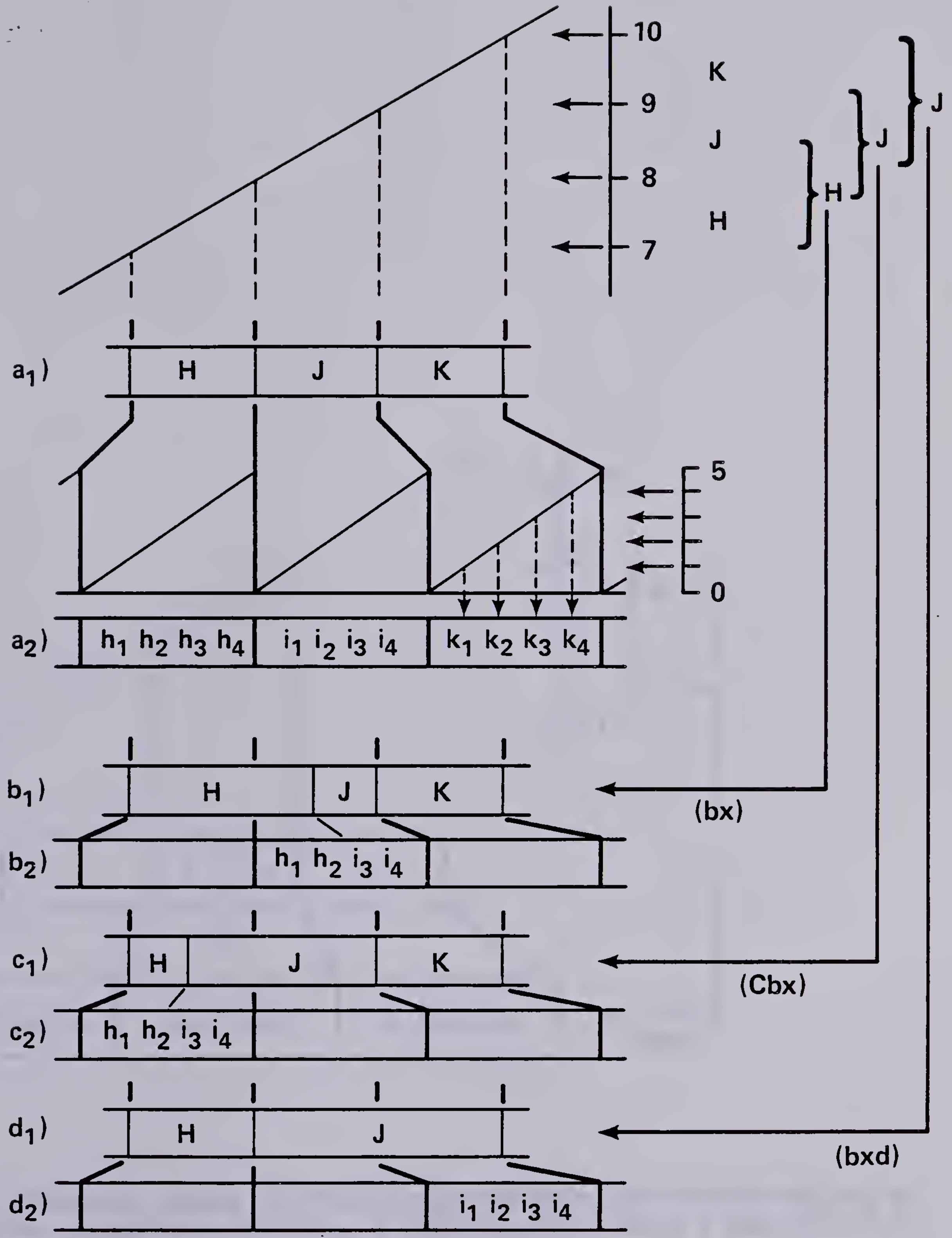


Figure 7-6. A model for the stepwise specification of body and segment pattern in insect embryos (from Sander, 1975b, reproduced by permission). (a-1) Local values of a primary axial gradient determine the segmental character of blastoderm cells (values 7-8 = segment H, 8-9 = segment J, 9-10 = segment K) and specify the position of physiological barriers. Within these barriers secondary gradients are established (values 0-5) which give the positional information within segments (a-2). (b)-(d) Erroneous reading of the local gradient value by the blastoderm cells (see brackets above right) would lead to a shifting in the pattern of determination of the blastoderm. In the cases when in spite of this the physiological barriers remain in their normal positions, partial segments (b-2), (c-2) or complete segments (d-2) would be transformed. If we label segments H, J, and K as Mesothorax, Metathorax, and the 1st Abdominal Segment, we see the transformations caused by three alleles of the Bithorax Complex (see brackets lower right).

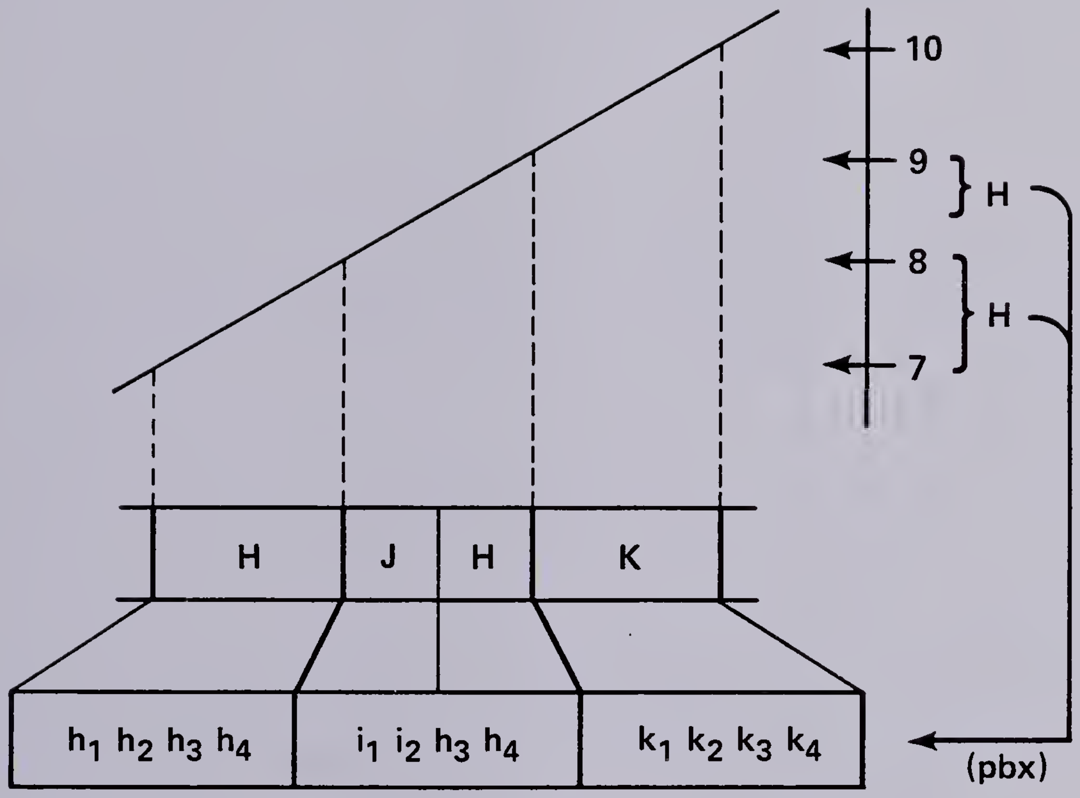


Figure 7–7. Erroneous reading of primary gradient (brackets above right) required to explain the *pbx*$^-$ mutant. Note that values 8 1/2–9 must be read as if they were 7–8, while values 8–8 1/2 are read correctly.

determined anterior and posterior compartments, one would predict the existence of a single "metathorax" gene rather than two genes which are compartment specific. This contradiction is never explicitly dealt with, as the interactions of the *en*, *bx*, and *pbx* genes in wild-type development are not discussed; rather, their interaction in mutant combination are considered, and I feel that they are misinterpreted. In the next section I will discuss the subject of mutant combinations with special reference to the interaction of *en* with genes of the Bithorax Complex.

VIII. Mutant Interactions

The five ways in which two different homeotic mutant transformations may be related are diagrammed in Figure 8–1. Two configurations, which I have called "disjunct" and "convergent" are of no concern to this discussion as the mutant effects are entirely independent and mutant combinations provide no additional information. A third configuration, the "reflexive" will also not be discussed because the resolution of any particular case must depend to a great extent upon the unique characteristics of the mutations involved, rather than the functions of the wild–type loci. This section therefore will discuss the expected transformation in mutant combinations which involve "linear" or "divergent" configurations.

A. Amorphic Combinations

Figure 8–2 shows three pattern units, A, B, and C, and their corresponding gene state sets. The state sets have been specifically chosen for illustrative purposes, but the reader may verify that the conclusions are general. Consider the effects of two mutations *x* and *y*, separately and in combination. It can be seen that in all the systems defined in section 4 -- combinatorial, cumulative, and epistatic –a mutation in *x* will transform A to B, a mutation in *y* will transform B to C, and the combination *x y* will result in a linear transformation of A to C..The combining of mutant effects in this fashion has been called "additivity" (Garcia–Bellido, 1975). I wish to point out that in linear configurations additivity is expected of mutant effects not only in combinatorial systems, but also when the wild–type genes interact cumulatively or epistatically.

When a domain may be transformed to different ranges by different mutants a divergent configuration arises. Combinations of such mutants have been called "paradoxical" (Kiger, 1976). Figure 8–3 shows the relationships of Figure 8–2 redrawn to include the effects of mutations in *y* on structure A. The unknown pattern unit is of interest. In a combinatorial system, it will have a unique identity I shall call D; A is thus transformed into B by *x* and D by *y*. The double mutant is thus a paradoxical genotype. The paradox is resolved by a transformation of A to C, a new pattern unit. Ideographic systems do not show similar behavior because of the redundancy in their codes. If Figure 8–3 is considered as a cumulative system, the state set of the unknown pattern unit is a

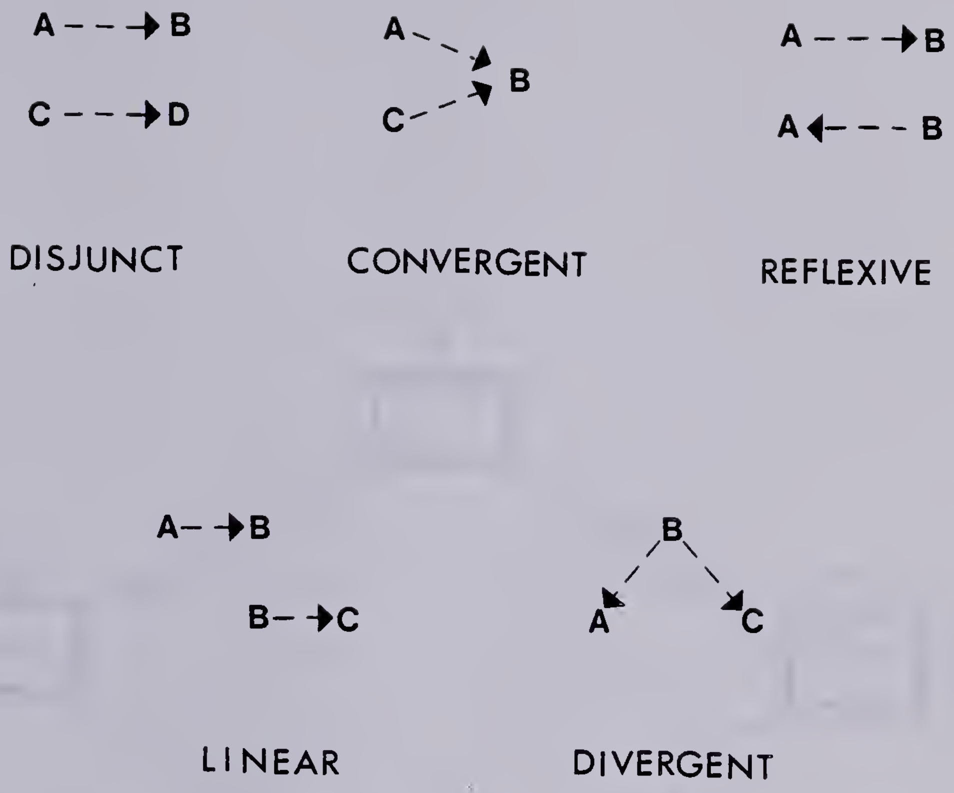


Figure 8-1. The five ways in which two different homeotic transformations may be related. Only linear and divergent configurations will be considered.

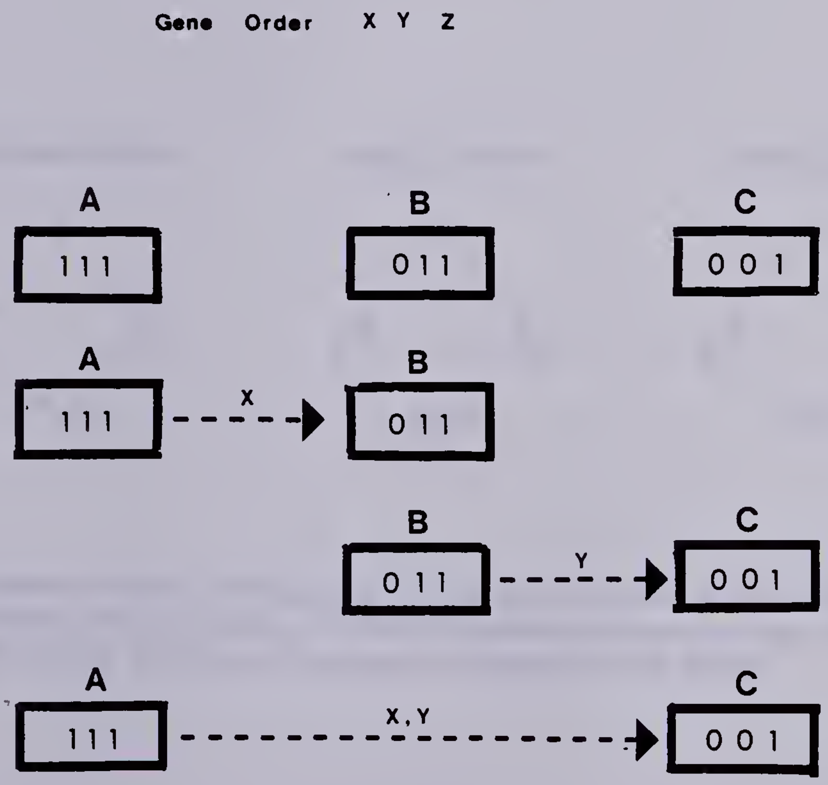


Figure 8-2. A linear transformation caused by two mutations, *x* and *y*. The results are equivalent whether the interaction system is combinatorial, cumulative, or epistatic.

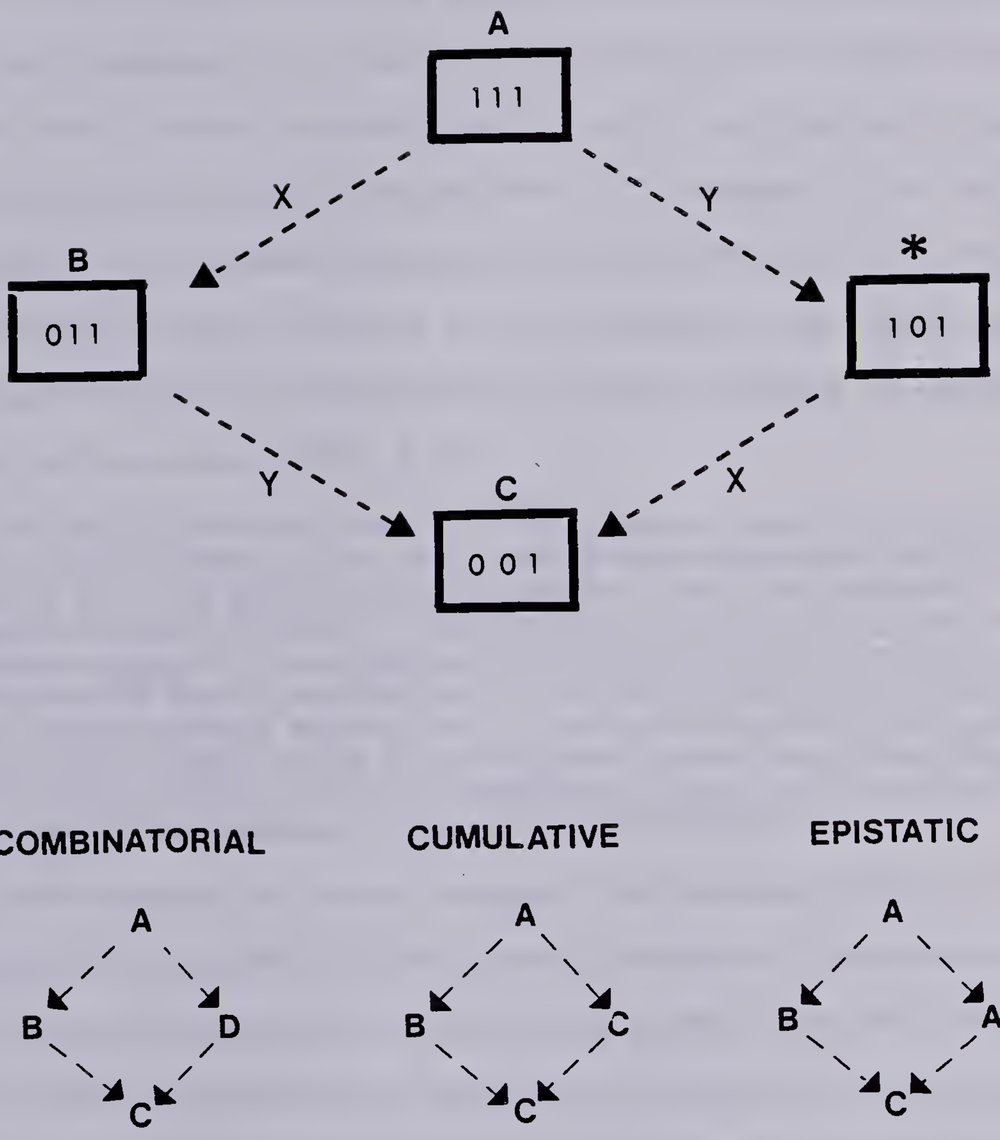


Figure 8-3. The relationships of Figure 8-2 redrawn to include the effects of mutation y on unit A. The starred unit will have a different identity in each of the three systems as shown below, allowing a distinction between systems to be made.

redundant code for unit C. Again, a paradoxical genotype is seen in the double mutant, because pattern unit A is transformed differently by each component. In a cumulative system, however, the double mutant combination has the same effect as one of the single mutants. If Figure 8–3 is considered as an epistatic system, the state set of the unknown pattern unit is a redundant code for unit A. The mutant combination therefore is not paradoxical. The *y* mutation does not transform unit A, but it alters the underlying state set, so that a mutation *x* now results in a transformation to C rather than to B.

On the basis of mutant combinations of *en* with *bx* and with *pbx* it has been proposed that *en* controls posterior development in the metathorax (Garcia–Bellido and Santamaria, 1972). Let us examine the nature of the argument in support of this conclusion, in light of the above analysis. It is not the double mutant phenotypes (see Figures 8–4 and 8–5) but their interpretation with which I disagree. I quote from Garcia–Bellido and Santamaria, 1972, p. 91:

> "In *en; bx*^{3} flies the posterior part of the metathorax remains unchanged.This indicates that the posterior part of the anlage does not derive from, or copy, the anterior part. The appearance of an anterior wing in the posterior metathorax indicates that the *en* transformation is anautonomous feature of the posterior region of these segments.In this case the effect of *en* is superimposed to that of *pbx*.The lack of function of *pbx*$^+$ in the metathorax leads to a developmental situation which permits us to detect the function of *en*$^+$, for when this fails, due to the *en* mutation, anterior wing structures develop in the posterior part of the metathorax. *These results indicate that en*$^+$ *also controls metathoracic development.*"(italics mine)

It is only this last statement with which I disagree. The phenotype of *en; bx*^{3} flies indicates that the *en* mutation has no effect on the posterior metathorax. The results are thus consistent with the pattern expected of an epistatic system (Figure 8–3). In *en* mutants the posterior haltere is unchanged, but the underlying state set is modified such that when *pbx*$^+$ is removed in the double mutant, the transformation is to anterior wing rather than to posterior wing. Thus, the results indicate that *en* does *not* control metathoracic determination.

B. Combinations Involving Neomorphs

The interactions of neomorphs with other mutations will vary depending upon the nature of the abnormal activation in each neomorph. Because of this, the phenotypes of mutant combinations involving neomorphs are instructive rather than diagnostic. That is, one cannot predict the specific phenotypes which will be associated with particular kinds

Figure 8–4. A haltere of genotype *en*^{1}; *bx*^{3}. Note the split scutellum and socketed bristles on the alula (arrows) in the mesothorax. Note also the unaffected posterior region of the haltere (cf. Figure 6–10).

Figure 8–5. A haltere of genotype *en; pbx*. Note the triple row bristles (TR) along the posterior margin while the anterior of the haltere is untransformed.

Fig. 8-4

Fig. 8-5

of control systems, and assign the wild-type genes to one system or another on the basis of phenotypic interactions, as can be done with amorphs. One can only observe the phenotypic results of mutant combinations and in some cases (alluded to in section 5) draw conclusions about the activation characteristics of the neomorph. The general question which may be asked concerns the nature of the neomorph domain: is the neomorph activated in a specific pattern unit because of the position of that unit in the field, or because of the determined state in that pattern unit? The question may be asked in two ways, which correspond to the linear and divergent configurations discussed above. In both cases the answers may be either definitive or ambiguous.

Consider a neomorph which causes a transformation of pattern unit B to unit C. Two results are possible when this neomorph is combined with a second mutation causing an A to B transformation (Figure 8-6). If only the autotypic B is transformed, one may conclude that activation of the neomorph is position specific rather than determined state specific. If both auto- and allotypic B's are transformed (i.e., if a linear transformation of A to B to C is observed) the results are inconclusive, for there exist two possibilities: (i) the neomorph may be activated by the determined state B, or (ii) the neomorph may be activated in a position specific manner, but its effect overridden by the control gene in pattern unit A.

The question whether the activation of a neomorph is position specific or determined state specific may be asked another way, by establishing a paradoxical genotype. Suppose the same neomorph transforming B to C were to be combined with a mutation transforming B to D (Figure 8-7). Two results are again possible. If the domain is still transformed to C, the neomorph may be said to be position specific. If the pattern unit is no longer transformed to C, either (i) abnormal activation of the neomorph is determined state specific, or (ii) it is position specific but is now epistatically overridden by the control gene for D.

Note that in both "linear" and "divergent" configurations, results are possible which would allow one to conclude unambiguously that the activation of a neomorph is governed by position rather than by determined state.

Combinations of *en* with the neomorphs *Hm* and *Cbx* allow a confirmation of the conclusions made from the earlier combination with *bx* and *pbx*. The *en* mutation causes

$$\begin{tikzcd}
A \arrow[r] & B \arrow[r, dotted, "?"] & C \\
& B \arrow[r] & C
\end{tikzcd}$$

Figure 8-6. See text for discussion.

$$\begin{tikzcd}
B \arrow[r] \arrow[d] & C \\
D \arrow[r, dotted, "?"] & C
\end{tikzcd}$$

Figure 8-7. See text for discussion.

$$\begin{tikzcd}
\mathrm{AMS} \arrow[r, "\mathrm{Hm}"] & \mathrm{AMT} \\
\mathrm{PMS} \arrow[u, "\mathrm{en}"] \arrow[r, "\mathrm{Hm}"] & \mathrm{PMT}
\end{tikzcd}$$

Figure 8-8. See text for discussion.

Figure 8–9. *Hm en*^{1}/+ *en*^{1}. Note the socketed bristles on the alula (arrow) but the lack of any *en* effect in the distal region (cf. Figure 6–20, see text for discussion).

Figure 8–10. *en; Cbx*. Note the triple row bristle at the posterior margin (cf. Figure 6–19, see text for discussion).

Fig. 8-9

Fig. 8-10

a transformation of the posterior to the anterior compartment in the wing, while *Hm* differentially activates the *bx* and *pbx* loci in the anterior and posterior compartments (Figure 6–20). The mutant combination *Hm en* thus establishes a potential linear configuration with regard to *bx* activation and a divergent configuration with regard to *pbx* activation (Figure 8–8). The resultant *Hm en* phenotype (Figure 8–9) shows that although the proximal wing exhibits anterior characteristics in the posterior region (note the socketed bristles in the alar lobe), only the autotypic anterior compartment is transformed to anterior metathorax, and the prospective allotypic anterior compartment is still transformed to the hybrid wing–haltere tissue characteristic of *pbx* activation. The results therefore demonstrate that in *Hm* the activation of *bx* and *pbx* are position specific and are not influenced by the state of the *engrailed* gene. The same conclusions may be drawn from the *Cbx en* combination. Figure 8–10 shows the wing of a *Cbx en* fly. The transformation of wing to haltere still occurs preferentially in the true posterior region (cf. Figure 6–17) in spite of the posterior to anterior transformation caused by *en* (note the isolated triple row bristle at the posterior margin typical of those found at the anterior margin in extreme *Cbx* phenotypes as in Figure 6–19).

IX. Deletions

The effects of deletions may differ from those of point mutants in two respects. First, deletions remove a physical structure which may be important in mediating regulative properties at the chromosomal level, and second, they may remove more than one control gene, and thus may have effects similar to combinations of point mutants as discussed in Chapter X. These two aspects will be discussed separately.

A. Loss of Physical Structures in Deletions

Transient exposure to ether vapour during the first six hours after oviposition may give rise to adult flies with phenocopies of various mutations in the Bithorax Complex (Gloor, 1947; Capdevila and Garcia–Bellido, 1974, 1978; Bownes and Seiler, 1977). The cellular blastoderm is the most sensitive stage, and *bithorax* phenocopies are by far the most common (Bownes and Seiler, 1977; Capdevila and Garcia–Bellido, 1974). Phenocopied tissue occurs in patches which have been shown to be clonal in origin (Capdevila and Garcia–Bellido, 1974). The lack of late effects of ether and the clonal nature of the phenocopies suggest that ether vapour interferes in some way with the activation of the metathoracic pathway rather than with its maintenance. Capdevila and Garcia–Bellido (1978) examined the effects of various heterozygous mutants in the Bithorax Complex upon phenocopy induction, and found that while point mutants had no effect, deletions and breakpoints at the *Ubx* locus doubled the frequency of induction. They proposed that activation of the Bithorax Complex is controlled by an interaction between inductor (sic) molecules and repressor molecules which bind at the *Ubx* locus. Ether vapour is assumed to perturb the inductor gradient such that enough repressor remains in the metathorax to inactivate the Bithorax Complex there, resulting in a phenocopy. Point mutants would not affect the frequency of phenocopy induction because the repressor binding site would remain intact, but deletions would effectively double the repressor:binding site ratio and thereby lower the degree of inductor perturbation necessary to induce a phenocopy. Their proposal is plausible, and a similar process may account for the recent results of Morata and Kerridge.

Morata and Kerridge (1981) made somatic clones of *Ubx* mutations and deficiencies at various times, with interesting results. In the second abdominal segment

and all segments more caudad *Ubx* clones differentiated as wildtype (see below). In the first abdominal segment *Ubx* clones were not observed, presumeably because thoracic cells have ceased dividing by the time the clones were segregated. In the anterior metathorax, the clones differentiated as anterior mesothorax, as expected. Not expected was the finding that clones in the posterior meso- and metathorax differentiated structures of the posterior prothorax if induced before seven hours, and of the posterior mesothorax if induced thereafter. They interpreted their results as indicating the existence of a gene within the proximal Bithorax Complex which is necessary only temporarily in early development to distinguish between prothoracic and mesothoracic developmental pathways. An alternative explanation which I favour would invoke a phenomenon similar to that used to explain the effects of deletions on phenocopy induction above. A large amount of evidence suggests that the prothorax-mesothorax developmental distinction is mediated by loci of the Antennapedia Complex (Lewis *et al.*, 1980a, b; Wakimoto and Kaufman, 1981). If the activity states of the Antennapedia Complex and the Bithorax Complex are controlled by the same positional cues, as seems likely, an early homozygous deficiency of the Bithorax Complex might influence the decision taken at the Antennapedia Complex by substantially altering the effective amount of a common repressor. *Ubx* clones would have no effect once the developmental decision at the Antennapedia Complex were made, however. The exact significance of the prothoracic transformation is not, as yet, clear.

B. Loss of Information Content in Deletions

In combinatorial systems the effects of deletions which remove more than one control gene may be easily calculated as the sum of the effects of single mutants in the genes deleted (see Chapter X).

In ideographic systems the effects of such deletions may differ depending upon the position of the deleted genes within the ordered series of all control genes. If the deleted genes comprise the high end of the ordered series the effects will be the same in epistatic and cumulative systems: only those pattern units whose control genes are deleted will be affected. Deletions which remove genes from the middle or lower end of the ordered system, however, will have differing effects in epistatic and cumulative

systems: in epistatic systems, as before, only those units whose control genes are deleted will be affected, while in cumulative systems, those units and all others controlled by genes higher in the ordering will be affected.

With respect to the Bithorax Complex, the *Ubx* clones generated by Morata and Kerridge are of interest in this regard. Such clones differentiated normally in abdominal segments 2 through 8, implying either that the proximal genes of the Bithorax Complex are the highest in ordering, or that the Complex as a whole is an epistatic system as regards the proximal loci, so that the removal of the lower components has no effect on segments controlled by genes higher in order. A number of consideratins strongly suggest that the latter interpretation is correct.

As Lewis notes (1978), the proximo–distal order of the Bithorax Complex loci along the chromosome corresponds more or less with the anterio–posterior position in the fly of the segments the loci control. In the proximal region it was deduced that the control genes for the metathorax are epistatic to those of the mesothorax (and, hence, higher in order), in part through the existence of neomorphic mutations (*Cbx*, *Hm*) which transform an anterior segment –mesothorax –– to one more posterior –– metathorax. A number of neomorphic mutants have been found in the distal region of the Complex (*Hab, Uab, Mcp,* see Lewis, 1978) which show similar caudad transformations, suggesting that the orientation of the ordered series is consistent throughout the Complex; the distal loci thus appear to be higher than the proximal loci in the order, and the Complex therefore appears to be an epistatic system.

In addition, Lewis (1978) has analysed the larval phenotypes of lethal heterozygotes for various partial deficiencies of the Complex and a complete deficiency. He found that when the proximal portion of the Complex was deleted the appropriate segments were transformed to mesothorax but the distal segments remained unchanged, a result equivalent to that of Morata and Kerridge. In addition, however, when distal portions were deleted the segments controlled by the remaining proximal loci appeared unchanged. The finding that deletions of both proximal and distal loci affect only their respective segments is compatible with an epistatic rather than a cumulative basis for Bithorax Complex gene interaction.

X. Conclusions

Because all previous models for the control of *Drosophila* development have each utilized a single system of genetic interaction and have often been less than fully defined or developed, they have led to conclusions which conflict with each other and with different aspects of the data. In this thesis I have defined rigorously various possible systems of gene interaction for the first time, and have investigated the expected properties of mutants in each. Further, I have argued on evolutionary and genetic grounds that in *Drosophila* development is controlled by a mixed system which incorporates elements of each hypothetic pure type. With this background, it has been possible to recognize the particular systems in which various wild-type genes operate on the basis of their mutant phenotypes. An analysis of the transformations observed in such mutants has led to further understanding in two major areas. As regards the control of gene activation, it has been found that the integration of information from two positional fields is required for the activation of the *bx* and *pbx* loci, and this in turn has led to a speculation as to the organization of the *bx*-region at the molecular level necessary to mediate the activation. As regards the control of determination, the concept of mixed systems has led to a coherent view of the effects of the *en* mutation , and a thorough investigation of the properties of interaction systems has revealed characteristics by which mutant combinations may properly be interpreted. According to this argument, erroneous conclusions have been imbedded in the literature on the basis of combinations of *en* with *bx* and *pbx*. Although these methods have here been applied for illustrative purposes only to an analysis of *engrailed* and the Bithorax Complex in this thesis, they should be generally applicable. The conclusions drawn are also not intended to be definitive -that will surely come only when the important loci have been characterized fully at the molecular level. Nonetheless, the molecular data -- the position and nature of mutants, the number and direction of transcripts, etc. -- will be of value only insofar as they explain how the genetic and developmental characteristics of the Complex are mediated. It is this latter aspect that I have attempted to clarify.

XI. Bibliography

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